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TESIS DOCTORAL

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EPIDEMIOLOGÍA MOLECULAR Y RESISTENCIA A LOS ANTIMICROBIANOS EN *Staphylococcus* spp. EN CENTROS SANITARIOS DE MALLORCA DURANTE LOS ÚLTIMOS 15 AÑOS (1999-2013)

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ANEXO 1: SOLUCIONES Y REACTIVOS

9. ANEXO 1: SOLUCIONES Y REACTIVOS

9.1 Electroforesis en campo pulsado

9.1.1 Soluciones de trabajo

Solución TE (TRIS, EDTA).

Reactivo en *stock*	**Volumen para 1 L**	**Concentración en la solución final**
EDTA (pH 7,6) 0,5 M	2 mL	1 mM
TRIS-HCl (pH 7,6) 2 M	5 mL	10 mM
Agua destilada	Hasta 1 L	

Solución TBE (TRIS, ácido bórico, EDTA) 5X.

Reactivo en *stock*	**Volumen para 1 L**
Ácido bórico	27,5 g
TRIS	54 g
EDTA (pH 7,6) 0,5 M	20 mL
Agua detilada	Hasta 1 L

Solución PIV.

Reactivo en *stock*	**Volumen para 30 mL**	**Concentración en la solución final**
TRIS-HCl (pH 7,6) 2 M	0,15 mL	10 mM
NaCl 5 M	6 mL	1 M
Agua destilada	24 mL	

Solución de lisis EC.

Reactivo en *stock*	**Volumen para 30 mL**	**Concentración en la solución final**
TRIS-HCl (pH 7,6) 2 M	90 µL	6 mM
NaCl 5 M	6 mL	1 M
EDTA (pH 7,6) 0,5 M	6 mL	100 mM
Brij® 58 5%	3 mL	0,5%
Desoxicolato Na 10%	600 µL	0,2%
Sarcosil 20%	750 µL	0,5%
RNAasa 10 mg/mL	60 µL	20 mg/mL
Lisozima 10 mg/mL	300 µL	100 mg/mL
Lisostafina 10 mg/mL	150 µL	50 mg/mL
Agua destilada	Hasta 30 mL	

Solución ESP.

Reactivo en *stock*	**Volumen para 30 mL**	**Concentración en la solución final**
EDTA (pH 9-9,5) 0,5 M	28,5 mL	0,5 mM
Proteinasa K 50 µg/mL	1,5 mg	50 µg/mL
Sarcosil 20%	1500 µL	1%

9.1.2 Reactivos, material e instrumental

Reactivos.

- Almacenados a temperatura ambiente:
  - TRIS-HCl (pH 7,6) 2 M.
  - NaCl 5 M.
  - EDTA 0,5 M (pH 7,6).
  - EDTA 0,5 M (pH 9-9,5).
  - Brij58® 5%.
  - Desoxicolato sódico 10%.
  - Sarcosil 20%.
  - Agarosa *low-melt.*
  - Agarosa Megabase®.
  - Bromuro de etidio 500 µg/mL.

- Almacenados a -20ºC:
  - RNAasa 10 mg/mL.
  - Lisozima 10 mg/mL.

- Lisostafina 10 mg/mL.
- Proteinasa K.
- Enzimas de restricción *Sma*I y *Apa*I, con sus tampones correspondientes.

Materiales.

- Tubos cónicos tipo *eppendorf* de 1,5 mL.
- Puntas de pipeta adaptadas al volumen necesario.
- Pipetas estériles desechables de 10 mL.
- Pipetas *Pasteur* de plástico estériles.
- Moldes para los bloques de agarosa (Bio-Rad).
- Tubos cónicos de tapón de rosca de 10 mL.
- Tubos cónicos de tapón de rosca de 50 mL (tipo *Falcon*).
- Gradillas para los tubos anteriores.
- Portaobjetos de vidrio.
- Cubreobjetos.
- Frascos de vidrio de 250 mL.
- Peine para gel de agarosa.
- Soporte para gel de agarosa.
- Probeta para un volumen total de 2 L.

Instrumental.

- Pipetas automáticas.
- Microcentrífuga.
- Microondas.
- Balanza.
- Baño de agua.
- Estufa de 37ºC.
- Estufa de 30 ºC.

- Equipo de campo pulsado (*Chef-DR® III System*, Bio-Rad).

9.1.3 Protocolo de trabajo

Preparación de los bloques de agarosa y lisis celular:

Día 0

- Descongelar cada cepa de estafilococo en una placa de agar sangre. La siembra se debe hacer por agotamiento, de forma que queden colonias bien aisladas.
- Incubar las placas en estufa a 37ºC durante 16-18 h.

Día 1

- Preparar en una gradilla un tubo cónico de 10 mL por cada cepa.
- Introducir en cada tubo cónico 5 mL de caldo BHI (***b****rain-****h****eart* ***i****nfusion*, caldo cerebro-corazón).
- Inocular una colonia aislada de estafilococo de cada cepa en un tubo cónico, previamente rotulado, que contiene el medio líquido.
- Incubar los tubos en la estufa a 35-37ºC durante 16-20 h.

Día 2

- En un tubo de tapón de rosca de 50 mL, preparar la solución PIV.
- Poner en una gradilla un tubo cónico tipo *eppendorf* estéril por cada cepa. Rotularlos.
- Depositar 1 mL del cultivo en medio líquido (suspensión de la colonia en caldo BHI) en su tubo *eppendorf* correspondiente.
- Centrifugar los tubos *eppendorf* durante 2 min, a 11.000 rpm.
- Decantar el sobrenadante.
- Inocular en cada *eppendorf* 1 mL de la solución PIV. Resuspender con la pipeta.
- Centrifugar otra vez los tubos tipo *eppendorf* durante 2 min en las condiciones anteriores.

- Decantar el sobrenadante dejando la menor cantidad de líquido posible.
- Inocular en cada tubo *eppendorf* 200 µL de solución PIV. Mezclar con la pipeta.
- Encender el baño de agua a 42ºC.
- Calentar el frasco de vidrio, que contiene la agarosa de bajo peso molecular (*low melt*) al 1,6%, previamente preparada, en el microondas hasta que comience a hervir. En este momento, comprobar que la agarosa esté bien disuelta (debe estar transparente). En caso de no haber agarosa *low melt* preparada, diluir en un frasco 0,8 g de agarosa *low melt* en 50 mL de tampón TBE 0,5X; calentar en el microondas hasta su completa disolución.
- Depositar el frasco de la agarosa *low melt* (una vez que esté bien disuelta) en el interior del baño de agua a 42ºC.
- Preparar las tiras de los moldes de agarosa, teniendo en cuenta que usaremos dos bloques de agarosa por cada cepa. Puesto que cada tira tiene 10 pocillos, cada una nos servirá para 5 cepas.
- Preparar al lado del baño de agua: una pipeta para un volumen de 200 µL, puntas de pipeta estériles para este volumen, las tiras de los moldes para la agarosa y la gradilla que contiene los tubos cónicos *eppendorf* con 200 µL de cada cultivo líquido resuspendido en la solución PIV.
- Con una pipeta, tomar 200 µL de la agarosa *low melt* y depositarla en el tubo *eppendorf* correspondiente. Mezclar varias veces con la pipeta la agarosa con el cultivo líquido.
- Rellenar dos moldes con la suspensión resultante (agarosa + cultivo líquido) por cada tubo *eppendorf*. Desechar el tubo usado.
- Repetir el mismo proceso para cada cepa.
- Dejar enfriar las tiras con los moldes de agarosa en nevera durante, al menos, 15 min.
- Preparar, en un tubo cónico tipo *Falcon* de 50 mL, la solución de lisis EC.
- Colocar en una gradilla un tubo cónico de 10 mL para cada cepa. Rotularlos con el número de la cepa.
- Inocular en cada tubo 1 mL de solución de lisis EC.
- Retirar el protector del adhesivo de la primera tira de los moldes. No tocar los bloques de agarosa con la mano.

- Transferir los dos bloques de agarosa de la cepa a su tubo de 10 mL correspondiente. Para ello empujar, con la ayuda de un asa de siembra de plástico, el bloque de agarosa (desde la parte contraria al adhesivo) hasta que el bloque caiga dentro de la solución de lisis EC del tubo.
- Repetir el mismo proceso para los demás bloques de agarosa.
- Incubar los tubos en la estufa de 35-37ºC durante un mínimo de 5 h y un máximo de 18 h.

Día 3

- Encender el baño de agua a 50ºC.
- Preparar, en un tubo cónico tipo *Falcon* de 50 mL, la solución ESP.
- Aspirar la solución de lisis EC de cada tubo con la ayuda de una pipeta *Pasteur* de plástico estéril.
- Añadir 1 mL de solución ESP a cada tubo.
- Incubar los tubos en el baño de agua a 50ºC durante 16-20 h.

Día 4

- Aspirar la solución ESP en cada tubo, utilizando una pipeta de plástico estéril.
- Realizar 5 lavados con 5 mL del tampón TE, con un intervalo aproximado de 30 min entre cada lavado.

Restricción del ADN y electroforesis de los fragmentos de restricción:

Día 1

- Preparar en una gradilla un tubo *eppendorf* por cada cepa de la que se vaya a realizar la restricción (con un máximo de 30). Rotularlos.
- Añadir a cada tubo 1 mL de agua destilada estéril.
- Sacar cuidadosamente, con la ayuda de un asa de siembra, uno de los dos bloques de agarosa de su tubo correspondiente por cada una de las cepas.
- Depositar el bloque de agarosa sobre un portaobjetos.

- Cortar con un cubreobjetos un fragmento de cada bloque de aprox. 1 mm de espesor.
- Transferir el fragmento a su tubo correspondiente.
- Volver a dejar el resto del bloque de agarosa en su tubo cónico correspondiente de 10 mL. Dejar los tubos otra vez en nevera.
- Incubarlos en estufa de 37ºC durante un mínimo de 10 min.
- Preparar la mezcla de restricción en un tubo de plástico de 10 mL, utilizando *Sma*I como enzima de restricción para *S. aureus*, o bien, *Apa*I para *S. hominis*.
- Retirar el agua destilada de cada uno de los tubos *eppendorf*.
- Transferir 200 µL de la mezcla de restricción a cada tubo.
- Incubarlos durante 16-20 h en estufa de 30ºC (para *Sma*I) o en estufa de 35-37ºC (para *Apa*I).

Día 2

- Eliminar con una pipeta la mezcla de restricción.
- Añadir a cada tubo *eppendorf* 1 mL de tampón TE.
- Incubarlos a 35-37ºC durante 1 h.
- Preparar 100 mL de agarosa megabase al 1% (*Certified Megabase Agarose*). Para ello, en un frasco de vidrio, introducir 1 g de agarosa megabase y luego 100 mL de TBE 0,5X.
- Calentar la mezcla en el microondas hasta su completa disolución.
- Montar el soporte del gel y depositarlo sobre una superficie plana.
- Colocar el peine de forma que quede recto.
- Añadir la agarosa megabase al molde cuando esté atemperada (aprox 50ºC).
- Dejar enfriar la agarosa durante 30 min hasta que solidifique.
- Añadir a la cubeta de electroforesis 2 L de agua destilada. Apretar los dos interruptores de la fuente de alimentación (*Power* y *Pump*). No encender todavía el refrigerador. Dejar el agua, al menos, unos 10 min para limpiar el sistema.

- Transcurrido este tiempo, apagar los dos botones de la cubeta de electroforesis. Eliminar toda el agua de la cubeta, colocando el grifo extraíble sobre la cubeta de electroforesis.
- Preparar, en una probeta de 2 L, el tampón TBE 0,5X. Añadir 200 mL del tampón TBE 5X sobre 1.800 mL de agua destilada.
- Añadir a la cubeta los 2 L del tampón TBE 0,5X. Encender los dos botones de la cubeta (*Power* y *Pump*) y el del refrigerador (*Cooling Module*).
- Pulsar el botón del refrigerador *Actual Temp* para ver la temperatura actual del tampón (el botón *Set Temp* indica la temperatura que se debe alcanzar, mientras que el botón *Actual Temp* indica la temperatura actual). El tampón debe alcanzar la temperatura de 14ºC (tarda unos 30 min en enfriarse).
- Calentar el frasco que contiene agarosa convencional al 0,8% en el microondas para disolverla. Dejarlo enfriar.
- Una vez que la agarosa megabase del molde se ha solidificado, retirar con cuidado el peine.
- Eliminar, con una pipeta *Pasteur*, el tampón TE del primer tubo *eppendorf*, que contiene el fragmento del bloque de agarosa.
- Golpear el tubo cónico sobre un portaobjetos para que el bloque de agarosa se levante del fondo.
- Introducir con cuidado (ayudándose con dos asas de plástico) el bloque de agarosa en el pocilllo correspondiente. Repetir lo mismo para el resto de los bloques.
- Sellar los pocillos con agarosa convencional al 0,8% (utilizando una pipeta).
- Comprobar que el tampón de la cubeta de electroforesis haya alcanzado los 14ºC.
- Desmontar con muchísimo cuidado, al lado de la cubeta, el soporte del gel.
- Colocar el gel (con el soporte negro) en el marco de la cubeta.
- Ajustar las condiciones del campo pulsado, usando los botones de la cubeta de electroforesis rotulados como:
    - "*INITIAL SW. TIME*" (pulso inicial): 1 segundo para *S. aureus*, 0,1 segundos para *S. hominis*.
    - "*FINAL SW. TIME*" (pulso final): 30 segundos para *S. aureus* y *S. hominis*.

- "*RUN TIME*" (duración): 23 h para *S. aureus*, 24 h para *S. hominis* (Sorlozano, 2010).

- Pulsar el botón de "*PAUSE. START RUN*", para iniciar la electroforesis.

Día 3

- Esperar hasta que acabe de correr el gel (23-24 h para *Staphylococcus*).
- Preparar en una bandeja la solución de bromuro de etidio: 300 µL de bromuro de etidio (0,5 µg/mL) en 300 mL de agua destilada.
- Sacar con cuidado el gel de la cubeta de electroforesis.
- Depositar el gel en la bandeja con bromuro de etidio.
- Mantener el gel en la bandeja (tapado con otra bandeja) durante un mínimo de 30 min.
- Colocar el gel sobre la bandeja, encender el transiluminador ultravioleta y poner el protector de plástico. Apretar el botón de la luz ultravioleta "*Trans* UV".
- Si se observan bandas, poner el gel en otra bandeja conteniendo unos 300 mL de agua destilada. Tapar también esta bandeja. Dejar el gel en la bandeja con agua unos 5-10 min.
- Volver a poner el gel sobre el transiluminador ultravioleta.
- Realizar la fotografía del gel. Guardar el archivo de imagen en el ordenador o en una memoria externa.

Resultados e interpretación:

- Cada cepa viene definida por un perfil o patrón de bandas de restricción.
- La comparación entre los diferentes patrones de bandas obtenidos establece la asociación epidemiológica entre las distintas cepas. Con ello determinaremos los diversos clones. Los criterios utilizados se basan en los descritos por Tenover (Tenover, 1995).

Controles utilizados en las ECP y nomenclatura:

Las cepas controles de SARM utilizadas fueron: tres cepas de los clones mayoritarios del HUSD (clones A, B, C) detectadas en estudios previos (Ruiz de Gopegui, 2005; Alcoceba, 2007), posteriormente tipificadas mediante MLST y PCR del SCC*mec* como ST125-MRSA-IVc (clon A), ST228-MRSA-I (clon B) y ST22-MRSA-IVh (EMRSA-15, clon C). También se emplearon dos cepas tipificadas del clon ST22-MRSA-IVh (EMRSA-15): DEN4561 (Faria, 2005) y HAR22 (Murchan, 2003). La cepa de referencia *S. aureus* NCTC 8325 (Tenover, 1995) se utilizó como marcador de peso molecular.

La nomenclatura de los clones de SARM se ha basado en la utilizada en nuestro primer estudio de ECP realizado en el Hospital de Bellvitge con aislados de SARM detectados en 1999-2000, junto con la nomenclatura estandarizada según el MLST y tipado del SCC*mec* (Enright, 2002).

9.2 Mezclas de reacción para PCR

Mezcla de reacción para MLST.

Reactivo	Reacción	Volumen (µL)
Buffer 10X (New England BioLabs)	1X	10,0
Mezcla dNTPs 100 mM (Bioline)	400 µM	0,4
arcC-Up 100 µM[a]	0,6 µM	0,6
arcC-Dn 100 µM[a]	0,6 µM	0,6
Taq DNA polimerasa 5 U/µL (New England BioLabs)	3 U	0,6
Agua destilada		85,8
ADN		2,0

[a]La composición de la mezcla es idéntica para las otras seis parejas de cebadores.

Mezcla de reacción para el complejo *ccr* tipos 1-3 del SCC*mec*.

Reactivo	Reacción	Volumen (µL)
Buffer II 10X (Applied Biosystems)	1X	5,0
$MgCl_2$ 25 mM (Applied Biosystems)	1,5 mM	3,0
Mezcla dNTPs 10 mM (Invitrogen)	300 µM	1,5
cA1 100 µM	0,6 µM	0,3
cA2 100 µM	0,6 µM	0,3
cA3 100 µM	1,0 µM	0,5
cB 100 µM	0,6 µM	0,3
Ampli*Taq* Gold DNA polimerasa 5 U/µL (Applied)	2,5 U	0,5
Agua destilada		36,6
ADN		2,0

Mezcla de reacción para el complejo *mec* de clase B.

Reactivo	Reacción	Volumen (µL)
Buffer II 10X (Applied Biosystems)	1X	5,0
$MgCl_2$ 25 mM (Applied Biosystems)	1,5 mM	3,0
Mezcla dNTPs 10 mM (Invitrogen)	300 µM	1,5
mA 100 µM	0,6 µM	0,3
ROrf2 100 µM	0,6 µM	0,3
Ampli*Taq* Gold DNA polimerasa 5 U/µL (Applied)	2,5 U	0,5
Agua destilada		37,4
ADN		2,0

Mezcla de reacción para el complejo *ccr5* del SCC*mec* tipo V.

Reactivo	Reacción	Volumen (µL)
Buffer II 10X (Applied Biosystems)	1X	5,0
$MgCl_2$ 25 mM (Applied Biosystems)	1,5 mM	3,0
Mezcla dNTPs 10 mM (Invitrogen)	300 µM	1,5
ccrC F2 100 µM	0,6 µM	0,3
ccrC R2 100 µM	0,6 µM	0,3
Ampli*Taq* Gold DNA polimerasa 5 U/µL (Applied)	2,5 U	0,5
Agua destilada		37,4
ADN		2,0

Mezcla de reacción para el subtipado del SCC*mec* tipo IV.

Reactivo	Reacción	Volumen (µL)
Buffer 10X (Bioline)	1X	5,0
$MgCl_2$ 50 mM (Bioline)	1,5 mM	1,5
Mezcla dNTPs 100 mM (Bioline)	1400 µM	0,7
J-IVa-F 100 µM	0,4 µM	0,2
J-IVa-R 100 µM	0,4 µM	0,2
J-IVc-F 100 µM	0,4 µM	0,2
J-IVc-R 100 µM	0,4 µM	0,2
J-IVh-F 100 µM	1,0 µM	0,5
J-IVh-R 100 µM	1,0 µM	0,5
Bio*Taq* DNA polimerasa 5 U/µL (Bioline)	2,5 U	0,5
Agua destilada		38,5
ADN		2,0

Mezcla de reacción para la detección de la LPV.

Reactivo	Reacción	Volumen (µL)
Buffer II 10X (Applied Biosystems)	1X	5,0
$MgCl_2$ 25 mM (Applied Biosystems)	1,5 mM	3,0
Mezcla dNTPs 10 mM (Invitrogen)	300 µM	1,5
mecA-F 100 µM	0,4 µM	0,2
mecA-R 100 µM	0,4 µM	0,2
nuc-1 100 µM	0,4 µM	0,2
nuc-2 100 µM	0,4 µM	0,2
luk-PV-1 100 µM	1,2 µM	0,6
luk-PV-2 100 µM	1,2 µM	0,6
Ampli*Taq* Gold DNA polimerasa 5 U/µL	3 U	0,6
Agua destilada		35,9
ADN		2,0

Mezcla de reacción para la detección del ACME.

Reactivo	Reacción	Volumen (µL)
Buffer 10X (Bioline)	1X	5,0
$MgCl_2$ 50 mM (Bioline)	1,5 mM	1,5
Mezcla dNTPs 100 mM (Bioline)	400 µM	0,2
arcA-F 100 µM	0,6 µM	0,3
arcA-R 100 µM	0,6 µM	0,3
Bio*Taq* DNA polimerasa 5 U/µL (Bioline)	2,5 U	0,5
Agua destilada		40,2
ADN		2,0

Mezcla de reacción para los genes *cfr*, *fexA*, *tet*(K), *tet*(M), *tet*(L), *ant(4')-Ia*, *dfrK*, *vga*(C).

Reactivo	Reacción	Volumen (µL)
Buffer 10X (Bioline)	1X	5,0
$MgCl_2$ 50 mM (Bioline)	1,5 mM	1,5
Mezcla dNTPs 100 mM (Bioline)	400 µM	0,2
Cebador-F 100 µM	0,6 µM	0,3
Cebador-R 100 µM	0,6 µM	0,3
Bio*Taq* DNA polimerasa 5 U/µL (Bioline)	2,5 U	0,5
Agua destilada		40,2
ADN		2,0

Mezcla de reacción para el gen ARNr 23S.

Reactivo	Reacción	Volumen (µL)
Buffer 10X (Bioline)	1X	10,0
$MgCl_2$ 50 mM (Bioline)	1,5 mM	3,0
Mezcla dNTPs 100 mM (Bioline)	400 µM	0,4
23S-F 100 µM	0,6 µM	0,6
23S-R 100 µM	0,6 µM	0,6
Bio*Taq* DNA polimerasa 5 U/µL (Bioline)	3 U	0,6
Agua destilada		82,8
ADN		2,0

ANEXO 2: ARTÍCULOS PUBLICADOS

10. ANEXO 2: ARTÍCULOS PUBLICADOS

infrequent dosage interval that permitted a longer time of virus replication in the absence of an adequate virus-static ganciclovir blood concentration. However, the goal of CMV prophylaxis is not to suppress CMV reactivation, but to avoid the development of CMV disease until a patient's specific T-cells are capable of coping with the virus [5]. As ganciclovir was administered only three times a week by a peripheral vein, outpatient treatment was possible. This allows cost savings and, in contrast to an oral virus-static therapy, is independent of the patient's compliance. The suggested targeted prophylactic approach is efficient, safe and easy to perform in an outpatient clinic setting. Future prophylactic therapies using new oral formulations of antiviral agents [14] should be compared to the regimen described in the present study.

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RESEARCH NOTE

Epidemiological relatedness of methicillin-resistant *Staphylococcus aureus* from a tertiary hospital and a geriatric institution in Spain

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ABSTRACT

From January 2000 to June 2002, 24 *Staphylococcus aureus* isolates were recovered from decubitus ulcers of patients in a geriatric institution, of which 17 (70.8%) were methicillin-resistant *S. aureus* (MRSA). Antibiotic resistance and DNA macrorestriction (pulsed-field gel electrophoresis; PFGE) patterns of the MRSA isolates were compared with a collection of 161 MRSA isolates from patients admitted to the institution's reference hospital. PFGE revealed the presence of

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five clonal types (found also in hospitalised patients) among the 17 MRSA isolates. The findings suggest nosocomial acquisition of the MRSA strains by five patients, with subsequent dissemination of the strains within the institution. The high rate of MRSA highlights the need for epidemiological analysis to control the dissemination of MRSA in long-term care facilities.

Keywords Epidemiology, methicillin resistance, MRSA, PFGE, *Staphylococcus aureus*, typing

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The introduction of methicillin into clinical practice was followed almost immediately by the first report in 1961 of methicillin-resistant *Staphylococcus aureus* (MRSA) strains [1]. MRSA soon disseminated through hospitals around the world and, in the 1980s, became one of the most important challenges in the treatment of nosocomial infections [2]. In Europe, the proportion of MRSA increased from <1% in 1980 to 30% in 1991 [3]. In Spain, the first MRSA outbreaks were detected simultaneously in 1988 in certain hospitals in Madrid and Barcelona [4,5]. However, MRSA was isolated seldom in Palma de Mallorca until the first outbreak was detected in June 1999, followed by endemicity.

The epidemiology of MRSA infections has shown changes recently in both clinical and microbiological aspects. The reservoir of MRSA is apparently shifting from the hospital setting to nursing homes [6,7], chronic-care facilities [8,9] and spinal cord injury units [10]. The present paper describes a molecular epidemiological study of MRSA isolates recovered from decubitus ulcers of geriatric patients in a large chronic-care facility in Mallorca. The epidemiological relatedness between these MRSA strains and those isolated in the reference hospital for this geriatric institution was also assessed.

The Hospital Universitario Son Dureta (HUSD) is the reference tertiary hospital of the Balearic Islands and has 980 beds. The Residencia de Pensionistas La Bonanova has 552 beds and is the main chronic-care facility of Mallorca. As part of an epidemiological surveillance study, MRSA isolates from geriatric patients with decubitus ulcers admitted to the geriatric institution from January 2000 to June 2002 were studied. Specimens were collected with sterile swabs and sent to the microbiology laboratory. *S. aureus* isolates were identified by conventional methods [11].

Antimicrobial susceptibility testing was performed by disk diffusion according to the guidelines of the National Committee for Clinical Laboratory Standards [12]. Antibiotic disks (Rosco Laboratories, Taastrup, Denmark) tested were oxacillin, penicillin, gentamicin, erythromycin, clindamycin, ciprofloxacin, rifampicin, trimethoprim–sulphamethoxazole, mupirocin, fusidic acid, teicoplanin and vancomycin. Additionally, latex agglutination was performed to confirm the presence of PBP2a (Slidex MRSA Detection kit; bioMérieux, Marcy l'Etoile, France).

Clonal relatedness of MRSA isolates was determined by pulsed-field gel electrophoresis (PFGE). Genomic DNA embedded in agarose blocks was digested after bacterial lysis with *Sma*I. Chromosomal DNA fragments were separated using the Chef-DR III system (Bio-Rad Laboratories, Richmond, CA, USA), with 23 h at 6 V/cm^2, and initial and final pulse times of 1 s and 30 s, respectively. PFGE band patterns were interpreted as described previously [13]. For comparative purposes, a collection of 161 previously typed MRSA isolates from patients admitted to HUSD during 1999 and 2000 was used. Nomenclature for MRSA clones in the present study was based on that established for the previous collection.

During the 30-month study period, 24 *S. aureus* isolates were recovered from the decubitus ulcers of 24 different patients admitted to the chronic care facility, of which 17 (70.8%) were MRSA. The demographics and clinical characteristics of patients with MRSA are shown in Table 1. Ten patients had been admitted previously to HUSD, of whom five had been admitted since 1999 (the date when the outbreak of MRSA began in HUSD). MRSA was isolated in pure culture from seven patients, and was associated with other pathogens from ten patients. Associated pathogens comprised *Pseudomonas aeruginosa* (five patients), *Escherichia coli* (three patients) and *Proteus mirabilis* (two patients).

PFGE revealed the presence of four different clones among the 17 MRSA isolates, one of which had two subtypes. Clone A was found in eight (47%) patients, clone B in one (6%) patient, clone C2 in five (29.5%) patients, and clone E in three

Table 1. Characteristics of patients with decubitus ulcers infected by MRSA

Characteristic	n
Demographics	
Mean age in years (range)	83 (75–94)
Sex (male/female)	5:12
Previous HUSD admissions	
Never	7 (42%)
Before 1999	5 (29%)
Since 1999	5 (29%)
Underlying disease	
Mobility limitation[a]	17 (100%)
Arterial hypertension	10 (59%)
Heart disease[b]	6 (35%)
Diabetes mellitus type 2	4 (23%)
Fever	8 (47%)
Number of ulcers	
Sole	15 (88%)
Multiple	2 (12%)
Location	
Foot	10 (59%)
Sacrum	7 (42%)
Hip	3 (18%)
Stage	
Stage II (epidermis, dermis)	4 (23%)
Stage III (subcutaneous tissue)	8 (47%)
Stage IV (muscle, bone)	2 (12%)
Unknown	3 (18%)
Therapy	
Topical[c]	15 (88%)
Systemic[d]	10 (59%)
No treatment	2 (12%)

[a]Including dementia, ischaemic stroke, osteoarthritis, hip fracture and Parkinson's disease.
[b]Including heart failure, coronary artery disease, and atrial fibrillation.
[c]Mupirocin (13 patients) or fusidic acid (two patients).
[d]Trimethoprim–sulphamethoxazole (five patients), gentamicin (two patients), vancomycin (two patients), and teicoplanin (one patient).
HUSD, Hospital Universitario Son Dureta.

(17.5%) patients. Two subtypes of clone E were found: clone E2 in two (11.5%) patients and clone E1 in one (6%) patient (Fig. 1). All of these clones were represented in the previous collection of 161 MRSA isolates from patients admitted to HUSD, although in different proportions (clone A, 63%; clone B, 20%; clone C2, 2.5%; and clone E, 1%). None of these clones was related to the Iberian clone described previously [14]. Interestingly, the five different clonal types of MRSA were detected in the five patients admitted to HUSD since 1999 (the time of the original outbreak).

All MRSA isolates were susceptible to vancomycin, teicoplanin, trimethoprim–sulphamethoxazole, rifampicin and fusidic acid, but were resistant to ciprofloxacin and erythromycin (except one isolate). Resistance to clindamycin (10.4%) and gentamicin (14.8%) was variable, even among isolates from the same clone. Six of the seven clindamycin-susceptible isolates showed an MLS_B-inducible phenotype. Clone A had four different patterns of susceptibility, with the most frequent (four patients) being characterised by resistance to erythromycin and clindamycin, and susceptibility to gentamicin. Clone C2

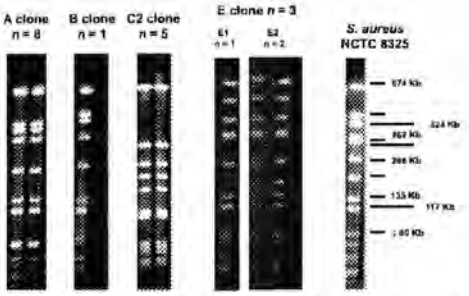

Fig. 1. *Sma*I PFGE macrorestriction patterns of the different MRSA clonal types. *Staphloccus aureus* NCTC 8325 was used as a molecular size marker. Lanes were obtained from two different gels and normalised using the bands produced by the NCTC control strain.

had two different patterns, with the most frequent (four patients) showing resistance to erythromycin, and susceptibility to gentamicin and clindamycin (with an MLS_B-inducible phenotype).

S. aureus infection is a significant cause of morbidity and mortality in elderly persons in hospitals, in geriatric institutions, and in the community [6]. *S. aureus* is the fourth most common hospital-acquired pathogen in older adults, following *E. coli*, *P. aeruginosa* and enterococci, and it accounts for 9% of all nosocomial infections in these patients [15]. In chronic care facilities, rates of infection are similar to those seen in hospitals [16]. Areas of chronic skin breakdown, such as decubitus ulcers, are common in hospitalised patients and nursing home residents. *S. aureus* is the microorganism isolated most frequently in aspirates from such ulcers [17]. Age-related changes in skin, together with an increased prevalence of peripheral vascular disease, diabetes mellitus and conditions that lead to diminished mobility, are associated with risk of skin infection in the elderly [16], as occurred in the patients in the present study. MRSA colonisation is common among nursing home residents (8–46% of residents), although reports of documented MRSA infection are relatively uncommon [16]. Limited studies suggest that most nursing home residents acquire MRSA during a hospital stay rather than in the nursing home [6]. It is thought that transmission of MRSA between nursing home residents is less frequent than in hospitalised patients [6].

The present study found an unexpectedly high rate of MRSA (70.8%) among *S. aureus* isolates

from decubitus ulcers of patients in this geriatric institution. Before 1999, no MRSA was detected in residents of this facility, and only a few sporadic cases were found in patients admitted to HUSD. Five different clonal types of MRSA were detected among the 17 patients of the geriatric institution, with the five patients who had been admitted to HUSD since 1999 each being infected by a different clonal type of MRSA. Since all these clones had been documented previously in patients admitted to HUSD, it seems reasonable to conclude that these patients acquired MRSA strains during their hospitalisation period. The fact that the other 12 patients had not been hospitalised previously in HUSD suggests that these MRSA clones may have spread subsequently between different patients within the geriatric institution. The high rate of MRSA found highlights the importance of epidemiological analysis in controlling the dissemination of MRSA in chronic care facilities, as in tertiary hospitals.

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RESEARCH NOTE

False synergy between vancomycin and β-lactams against glycopeptide-intermediate *Staphylococcus aureus* (GISA) caused by inappropriate testing methods

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ABSTRACT

The combination of vancomycin and β-lactams is often considered synergistic and has been recommended for the treatment of glycopeptide-intermediate *Staphylococcus aureus* (GISA) infections. In this study, the combination of vancomycin or

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ORIGINALES

Consolidación de un clon multirresistente de *Staphylococcus aureus* no relacionado con el clon Ibérico en un hospital de Mallorca

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INTRODUCCIÓN. El primer brote de *Staphylococcus aureus* resistente a meticilina (SARM) en nuestro hospital se detectó en 1999. Recientemente se ha observado un incremento de la multirresistencia asociada a SARM, lo que ha motivado la realización de un estudio de epidemiología molecular para averiguar las bases de esta tendencia.

MÉTODOS. Se documentaron todos los pacientes con aislamiento de SARM en muestras clínicas ingresados entre julio de 2002 y junio de 2003. El perfil de sensibilidad antibiótica se determinó mediante difusión con discos. El estudio de epidemiología molecular se realizó por electroforesis de campo pulsado (PFGE). Se han comparado los resultados obtenidos con datos históricos de 1999-2000.

RESULTADOS. Se aisló SARM en 110 pacientes (30% de los pacientes con aislamiento de *S. aureus*). Mediante PFGE se detectaron tres clones mayoritarios (93% de pacientes). Estos clones estaban ya presentes en el estudio previo realizado en 1999-2000, aunque con un cambio en su distribución. Mientras que en 1999-2000 el mayoritario fue el clon A (clon A, 63%; clon B, 20%), actualmente ha sido desplazado por el clon B (clon B, 58%; clon A, 19%). Ninguno de los clones mayoritarios está relacionado con los cinco clones pandémicos (incluido el Ibérico) pero dos de ellos parecen relacionados con los dos clones más frecuentemente encontrados actualmente en España. El nuevo clon mayoritario presentó de forma uniforme resistencia a ciprofloxacino, eritromicina, clindamicina y gentamicina.

CONCLUSIÓN. En los últimos años se ha producido un desplazamiento de un clon de SARM inicialmente predominante por otro multirresistente no relacionado con el clon Ibérico.

***Palabras clave:* Epidemiología molecular. *Staphylococcus aureus*. Resistencia a la meticilina.**

Consolidation of a *Staphylococcus aureus* multiresistant clone not related to the Iberian in a hospital from Mallorca

INTRODUCTION. Methicillin-resistant *Staphylococcus aureus* (MRSA) was seldom isolated in our hospital until the first outbreak in 1999. A recently documented increase in antibiotic multiresistance in MRSA strains in our setting prompted the design of this molecular epidemiology study to investigate the basis for this tendency.

METHODS. All MRSA isolates from clinical samples of patients admitted from July 2002 to June 2003 were studied. Susceptibility testing was performed by disk diffusion. Clonal relatedness of MRSA isolates was determined by pulsed-field gel electrophoresis (PFGE). Results were compared with data from MRSA isolates from patients admitted to the hospital in 1999-2000.

RESULTS. MRSA was isolated in 110 patients (30% of patients with *S. aureus*-positive cultures). PFGE detected three major clones (in 93% of patients), all of which had been present in 1999-2000, although in different proportions. Whereas the predominant clone in 1999 was clone A (clone A 63%, clone B 20%), clone B was now found to predominate (clone B 58%, clone A 19%). None of these major clones were related to the five pandemic clones, including the Iberian clone, but two of them seemed to be related to the two most prevalent clones in Spain at this time. The new predominant clone was more resistant than the others, and showed uniform resistance to ciprofloxacin, erythromycin, clindamycin, and gentamicin.

CONCLUSION. In recent years, a formerly predominant MRSA clone has been replaced by a multiresistant *S. aureus* clone that is unrelated to the Iberian clone.

***Key words:* Molecular epidemiology. *Staphylococcus aureus*. Methicillin resistance.**

Introducción

En 1961, pocos meses después de la introducción de la meticilina en la práctica clínica, se detectaron en el Reino Unido las primeras cepas de *Staphylococcus aureus* resistentes a meticilina (SARM)[1]. A principios de la década de 1960, las cepas de SARM se diseminaron rápidamente a lo largo de varios hospitales europeos, y se

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convirtieron, a partir de los años 1980, en uno de los principales patógenos responsables de infecciones nosocomiales, sobre todo en las unidades quirúrgicas o de cuidados intensivos[2,3].

En España, el primer brote intrahospitalario por este microorganismo se describió en 1981 en un hospital de San Sebastián[4,5]. La mayoría de los brotes de SARM entre 1989-1995 estaban producidos por el llamado clon Ibérico, detectado por primera vez en el Hospital Universitario de Bellvitge de Barcelona en 1989[6]. Dicho clon se diseminó a otros países, como Portugal, Escocia, Italia, Bélgica, Alemania, y a un hospital de Nueva York[7,8]. Las cepas de este clon se caracterizaron por presentar resistencia a múltiples grupos de antibióticos: macrólidos, tetraciclinas, aminoglucósidos y quinolonas, y ser sensibles a los glucopéptidos y al cotrimoxazol. A partir de mediados de la década de 1990, en el conjunto de España, ha ido disminuyendo el predominio del clon Ibérico, siendo sustituido por otros clones de SARM sensibles a más antibióticos, como muestran los datos de un reciente estudio de ámbito nacional realizado conjuntamente por el Grupo de Estudio de Infección Hospitalaria (GEIH), Grupo de Estudio de Mecanismos de Acción y Resistencia a Antimicrobianos (GEMARA) y la Red Española de Investigación en Patología Infecciosa (REIPI)[9].

En el Hospital Universitario Son Dureta de Palma de Mallorca, el SARM fue rara vez aislado hasta junio de 1999, cuando se detectó el primer brote de SARM, seguido de una endemia moderada.

Recientemente se ha constado un aumento en la frecuencia de cepas de SARM multirresistentes, lo que motivó la realización de un estudio de epidemiología molecular de los aislados recuperados en muestras clínicas de los pacientes ingresados en nuestro hospital entre el 1 de julio de 2002 y el 30 de junio de 2003. Además, los resultados obtenidos se han comparado con datos históricos de 1999-2000.

Métodos

El Hospital Son Dureta es un hospital terciario con aproximadamente 900 camas, de referencia para toda la Comunidad de las Islas Baleares. Está compuesto por dos pabellones de hospitalización separados, aunque situados en el mismo recinto: el hospital general y el hospital materno-infantil.

A partir de los registros microbiológicos se han documentado todos los pacientes con aislamiento de SARM en muestras clínicas que han ingresado en nuestro hospital entre el 1 de julio de 2002 hasta el 30 de junio de 2003. No se incluyeron en el estudio las cepas aisladas de cultivos de vigilancia del estado de portador (exudados nasales, axilares y/o inguinales). La identificación de los aislados de *S. aureus* se realizó por métodos convencionales[10].

El perfil de sensibilidad antibiótica (oxacilina, penicilina, eritromicina, clindamicina, ciprofloxacino, gentamicina, cotrimoxazol, vancomicina, teicoplanina, rifampicina, mupirocina y ácido fusídico) se determinó mediante el método de difusión con discos (Rosco, Taastrup, Dinamarca) siguiendo los criterios del National Committee for Clinical Laboratory Standards (NCCLS)[11]. Además, se realizó aglutinación con látex de los aislados de SARM para confirmar la presencia de PBP2' (Slidex MRSA Detection, bioMérieux, Marcy l'Etoile, Francia).

La relación clonal de los aislados de SARM se determinó mediante electroforesis en campo pulsado (PFGE), utilizando SmaI como enzima de restricción. La electroforesis se realizó utilizando el aparato Chef-DR® III (Bio-Rad, Richmond, EE.UU.) con las siguientes condiciones: pulso inicial de 1 s, pulso final de 30 s, tiempo de electroforesis 23 h a 6 V/cm. Los patrones de bandas de la PFGE se interpretaron según los criterios de Tenover[12]. Como controles se utilizaron los clones de SARM predominantes en un estudio multicéntrico nacional P_1 y Q_1[9] y los clones pandémicos Ibérico (ATTC BAA-44)[7], Brasileño (ATTC BAA-43)[13], Húngaro (ATTC-BAA 39)[14], Nueva York/Japón (ATTC BAA-41)[15] y Pediátrico (ATTC BAA-42)[16]. La cepa de *S. aureus* NCTC 8325[12] se utilizó como marcador de peso molecular.

Para el estudio comparativo se utilizó una colección de 161 cepas de SARM, previamente tipificadas, de pacientes ingresados en nuestro hospital durante los años 1999 y 2000 (datos no publicados). La nomenclatura de los clones de SARM de nuestro trabajo se basó en la utilizada en este estudio.

TABLA 1. **Distribución de los aislados de SARM por tipo de muestra**

Tipo de muestra	Número (%)
Secreciones respiratorias	51 (46,4)
Exudados	36 (32,7)
Hemocultivo	19 (17,3)
Catéter	14 (12,7)
Líquidos estériles	7 (6,4)
PAAF, biopsia	3 (2,7)
Total	***130 (100,0)***

SARM: *Staphylococcus aureus* resistente a meticilina; PAAF: punción-aspiración por aguja fina.

Resultados

Durante el período del estudio (julio de 2002-junio de 2003), el porcentaje de aislados de SARM en muestras clínicas con respecto al total de los aislados de *S. aureus* fue del 38%; este porcentaje descendió al 30% al referirlo al número de pacientes distintos con *S. aureus* en una muestra clínica. Se aisló SARM en 110 pacientes distintos: en 86 de ellos (78%) se detectó en una única muestra, mientras que en 24 (22%) se aisló en más de una. Las muestras más frecuentes fueron las secreciones respiratorias (51 pacientes; 46%), exudados (36 pacientes; 33%), hemocultivos (19 pacientes; 17%), y catéteres (14 pacientes; 13%) (tabla 1).

Mediante PFGE se detectaron tres clones mayoritarios denominados A, B y C, que comprendían el 93% de los pacientes, así como ocho clones minoritarios, cada uno de éstos en un único paciente. El clon A se encontró en 21 (19,1%) pacientes, el clon B en 63 (57,3%) pacientes y el clon C en 18 (16,4%) pacientes, cada uno de ellos con varios subtipos. El patrón de bandas del campo pulsado en los tres clones más frecuentes se muestra en la figura 1.

La distribución por servicios de los clones de SARM se resume en la figura 2. El clon B fue el más frecuente en pacientes de unidades de cuidados intensivos (76%) y de áreas quirúrgicas (69%), mientras que en los servicios médicos la frecuencia de los clones A y B fue similar.

Estos tres clones mayoritarios estaban ya presentes en el estudio previo de 1999-2000, aunque en menos de 2 años ha habido un importante cambio en su distribución. Mientras que en 1999-2000 predominaba el clon A (63% de los aislados), actualmente éste ha sido desplazado por el clon B (57% de los aislados) (fig. 3). Este desplazamiento coincide con un aumento del porcentaje de resistencia a

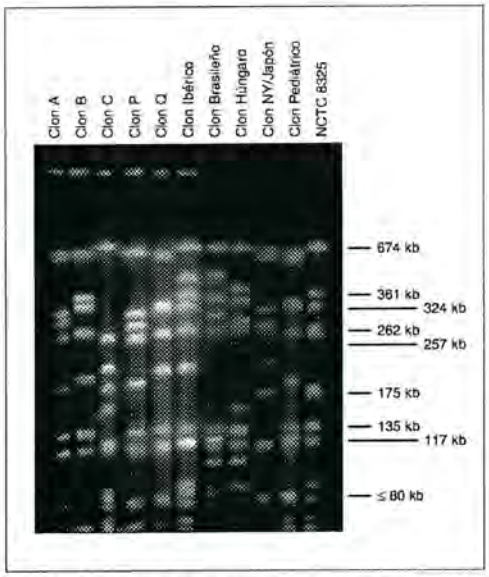

Figura 1. Comparación de los patrones de macrorrestricción con SmaI de los tres clones mayoritarios con diferentes clones de SARM representativos a nivel nacional y mundial. Carriles 1 a 11: clon A, clon B, clon C, clon P_1[9], clon Q_1[9], clon Ibérico[7], clon Brasileño[13], clon Húngaro[14], clon Nueva York/Japón[15], clon Pediátrico[16], *Staphylococcus aureus* NCTC 8325[12] (utilizado como marcador de peso molecular).

meticilina que pasó del 21% (por pacientes distintos) en el estudio previo (1999-2000) al 30% en el estudio actual (2002-2003).

Ninguno de los tres clones mayoritarios está aparentemente relacionado genéticamente con los cinco clones de SARM pandémicos descritos[17]: los clones Ibérico[6,7], Brasileño[13], Húngaro[14], Nueva York/Japón[15] y Pediátrico[16] (fig. 1). Asimismo, ninguno de los tres clones está directamente relacionado con los dos más frecuentemente encontrados en el estudio de ámbito nacional, aunque los clones A y B presentan cierta similitud con los clones P y Q[9], respectivamente.

La resistencia general de todos los aislados de SARM del estudio y de los tres clones mayoritarios se muestra en la tabla 2. Ninguna cepa de SARM fue resistente a la vancomicina, teicoplanina o rifampicina. Únicamente el 4,5% fueron resistentes a cotrimoxazol, mupirocina y a ácido fusídico. La mayoría fueron resistentes a ciprofloxacino (98%) y a eritromicina (90%). La resistencia a la gentamicina fue variable (61%). Con respecto a la clindamicina, 87 aislados (79%) presentaban una resistencia MLS_B *(macrolide, lyncosamide, streptogramin B)* constitutiva, mientras que 11 (10%) mostraban un fenotipo MLS_B inducible, la mayoría de los cuales (10 de los 11) pertenecían al clon C. El nuevo clon mayoritario (clon B) fue significativamente más resistente que los otros, presentando de forma uniforme resistencia a la eritromicina, clindamicina, ciprofloxacino y gentamicina.

TABLA 2. **Resistencia antibiótica general y por clones de los aislados de SARM**

Antibiótico	Número de aislados de SARM resistentes (%)			
	SARM totales (n = 110)	Clon A (n = 21)	Clon B (n = 63)	Clon C (n = 18)
Eritromicina	99 (90)	15 (71)	62 (98)	16 (89)
Clindamicina				
MLS_B constitutivo	87 (79)	15 (71)	61 (97)	5 (28)
MLS_B inducible	11 (10)	0 (0)	1 (2)	10 (56)
Ciprofloxacino	108 (98)	21 (100)	63 (100)	18 (100)
Gentamicina	67 (61)	2 (10)	61 (97)	1 (6)
Cotrimoxazol	5 (5)	0 (0)	3 (5)	0 (0)
Vancomicina	0 (0)	0 (0)	0 (0)	0 (0)
Rifampicina	0 (0)	0 (0)	0 (0)	0 (0)
Mupirocina	5 (5)	2 (10)	3 (5)	0 (0)
Ácido fusídico	5 (5)	2 (10)	1 (2)	1 (6)

SARM: *Staphylococcus aureus* resistente a meticilina; MLS_B: *macrolide, lyncosamide, streptogramin* B.

Discusión

Staphylococcus aureus resistente a la meticilina continúa siendo uno de los principales patógenos nosocomiales. Una vez que se introduce en el hospital, por lo general se llega a situaciones de endemicidad, a pesar de las medidas de control de la infección[8]. En los últimos años, el reservorio de SARM ha pasado de detectarse inicialmente en los grandes hospitales, a las residencias geriátricas y centros de larga estancia, suponiendo un riesgo de transmisión a la comunidad. Un estudio realizado por nuestro grupo en *S. aureus* procedentes de úlceras de decúbito de pacientes ingresados en una residencia geriátrica para la que nuestro hospital es el centro de referencia mostró el 70% de resistencia a la oxacilina en los aislados de *S. aureus*. El análisis de PFGE reveló, entre los 17 aislados de SARM de pacientes distintos, la presencia de cuatro clones, todos ellos encontrados también en pacientes ingresados en nuestro hospital[18].

En el Hospital Son Dureta, el primer brote de SARM se detectó en junio de 1999, 10 años más tarde que en otros hospitales de Madrid y Barcelona. Inicialmente se aisló en pacientes ingresados en las unidades de cuidados intensivos y traumatología, extendiéndose después a la mayoría de las plantas del pabellón de hospitalización general. Curiosamente, hasta la fecha, no se ha encontrado ningún aislado de SARM en pacientes del pabellón materno-infantil. La situación epidémica inicial de nuestro hospital se transformó en una endemia moderada.

Durante el período del estudio (julio de 2002-junio de 2003), el porcentaje de SARM con respecto al total de los aislados de *S. aureus* fue del 38%, mientras que el porcentaje de SARM en relación al número de pacientes con aislamiento de *S. aureus* fue del 30%. Este porcentaje es superior al encontrado en 36 hospitales españoles en el año 2002 según el European Antimicrobial Resistance Surveillance System (EARSS)[19], que fue del 23%, y también al del estudio español de los grupos GEIH/GEMARA/REIPI realizado en junio de 2003 en el que participaron 66 hospitales, con una frecuencia media de SARM del 21,8%[20]. Nuestra tasa de SARM es similar a la encontrada en otros países mediterráneos como Italia (38%) y Francia (33%) y algo inferior a la del Reino Unido (44%)[19].

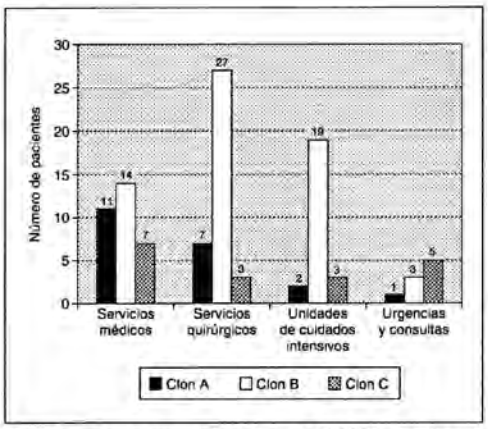

Figura 2. Distribución de los distintos clones de SARM del Hospital Universitario Son Dureta por servicios.

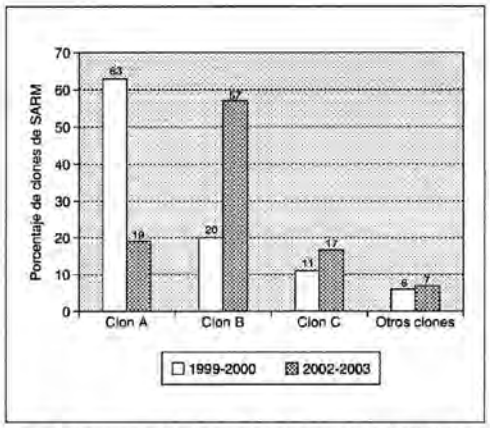

Figura 3. Comparación de la distribución de los clones de SARM del HUSD en los períodos 1999-2000 y 2002-2003.

En este trabajo, la gran mayoría de SARM (93%) detectados recientemente pertenecían únicamente a tres clones. En este sentido, concuerda con otros que señalan que las cepas de SARM hospitalarias corresponden a unos pocos clones. Así, por ejemplo, en un estudio realizado en más de 3.000 aislados de SARM procedentes de hospitales de Europa y América, casi el 70% de los aislados pertenecía a uno de los cinco clones pandémicos[17]. En otro trabajo realizado en 217 aislados de SARM de 12 hospitales de varios estados de Estados Unidos, el 79% pertenecía a dos únicos clones. Por último, en el estudio español de GEIH/GEMARA/REIPI el 80% de las cepas pertenecía a dos clones mayoritarios, que llamaron clon P y clon Q[9].

En el estudio de la sensibilidad antibiótica, nuestros aislados tenían una alta resistencia al ciprofloxacino (98%), eritromicina (90%), clindamicina (79%) y gentamicina (61%). La resistencia a la mupirocina (5%) fue más baja que la informada en otros centros (22% en el estudio español[9]) probablemente debido al uso del ácido fusídico en vez de la mupirocina como primera elección en el tratamiento de la descontaminación de los portadores de SARM. La cifra de resistencia al ácido fusídico es baja (5%), pero se desconocen los datos de resistencia a este antibiótico a nivel nacional.

Si se comparan los resultados de 2002-2003 con el estudio previo de 1999-2000, se puede observar que en los últimos años se ha producido un acusado desplazamiento del clon inicialmente predominante (clon A) por otro con mayores resistencias asociadas (clon B). El nuevo clon mayoritario presenta resistencia uniforme al ciprofloxacino, eritromicina, clindamicina y especialmente a gentamicina, característica que le diferencia del resto de los clones mayoritarios en los que el porcentaje de resistencia a este antibiótico únicamente fue del 10 y del 6% para los clones A y C, respectivamente.

Ninguno de los tres clones mayoritarios está aparentemente relacionado genéticamente con los cinco clones de SARM pandémicos descritos. No obstante, si bien el clon C es completamente distinto del resto, los clones A y B de este estudio presentan importantes similitudes con los clones P y Q, respectivamente (fig. 1), quizá reflejando la presencia de una línea ancestral común que debe evaluarse con técnicas con mayor capacidad para establecer relaciones clonales a largo plazo como el MLST *(multilocus sequence typing)*. El clon C parece sin embargo mostrar un perfil de restricción similar al clon EMRSA-15 común en hospitales británicos[21].

Mientras que la tendencia nacional es una clara disminución de las resistencias asociadas en las cepas de SARM[9], en nuestro hospital ha ocurrido justamente lo contrario: el desplazamiento de un clon moderadamente resistente por otro multirresistente (no relacionado con el clon Ibérico) en un período de tiempo relativamente corto. Este desplazamiento determina el hecho preocupante de que las resistencias asociadas a SARM en nuestro medio sean muy superiores a la media nacional. En este sentido destacan las resistencias a la clindamicina y la gentamicina que doblan la media nacional (79% frente a 38% y 61% frente a 26% para la clindamicina y la gentamicina, respectivamente)[9].

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Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Majorcan hospitals: high prevalence of the epidemic clone EMRSA-15

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ABSTRACT

Clinical isolates (n = 389) of methicillin-resistant *Staphylococcus aureus* (MRSA) recovered from 371 patients between January 2003 and June 2004 at the three major public hospitals on the island of Majorca, Spain were studied. The clonal relatedness of MRSA isolates was determined by pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I. During the study period, MRSA was found in 31% of patients with *S. aureus*-positive cultures. PFGE analysis identified three predominant clones, affecting 94% of the patients. The three clones had been detected since 1999 in one hospital, and were designated as clones A, B and C. Whereas clones A and B (multidrug-resistant) were related to the two most prevalent clones in Spain at this time, clone C was identical to EMRSA-15, currently one of the most common MRSA clones in UK hospitals and also detected in other countries, but rarely in Spanish hospitals. This imported epidemic clone was detected in *c.* 10% of patients admitted to one of the three hospitals in 2002, but its prevalence has increased significantly (32% of the patients investigated in the three hospitals in the present study), and this clone also accounted for 44% of the isolates from non-hospitalised patients. Even though EMRSA-15 showed the least multidrug resistance of the three major clones, it was apparently more virulent, since it was associated significantly (p 0.001) with bacteraemia, and positive blood cultures were documented for 21% of the patients infected by this clone, compared with only 10% and 7% of patients infected with clones A and B, respectively.

Keywords Clones, epidemiology, Majorca, methicillin-resistant *Staphylococcus aureus*, pulsed-field gel electrophoresis, virulence

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were identified soon after the introduction of methicillin into clinical practice [1,2]. Since the first outbreaks of infection caused by MRSA in Europe during the early 1960s [3], this pathogen has spread worldwide, not only throughout the hospital environment, but also among community patients without exposure to healthcare systems. Furthermore, the incidence of MRSA is increasing, despite the development of infection control programmes in many countries. Molecular epidemiology studies have shown that the current MRSA pandemic is the result of the global dissemination of a few highly successful clones [4,5].

In Spain, the first nosocomial outbreak of this pathogen was detected in 1981 [6,7], but MRSA was not a serious problem until the first outbreaks were detected in major Spanish cities at the end of 1989 [8,9]. Most MRSA outbreaks in Spain between 1989 and 1995 were caused by the 'Iberian' clone, which was first detected in 1989 in Barcelona [10], and which showed resistance to most antibiotic groups (i.e., macrolides, tetracyclines, aminoglycosides and quinolones). However, in the mid-1990s, this clone was progressively supplanted by other MRSA clones associated with less multidrug resistance [11,12]. A Spanish surveillance study performed in 2002

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showed a progressive increase in the proportion of MRSA isolates (from 1.5% in 1986 to 31.2% in 2002), and an alarming number of methicillin-resistant isolates were recovered from outpatients; thus, while <5% of community-acquired *S. aureus* isolates in 1994 were methicillin-resistant, 17.8% were methicillin-resistant in 2002 [13].

The first MRSA outbreak on the island of Majorca was described in June 1999; since then the epidemiology of MRSA infections has changed in terms of both clinical and microbiological aspects. The aims of the present work were to analyse recent data concerning the molecular epidemiology and susceptibility of the different MRSA clonal types circulating in Majorcan hospitals, and to compare these results with those of previous local and national studies in Spain, in order to describe the evolution of the epidemiology of this pathogen on Majorca in recent years.

MATERIALS AND METHODS

Patients and clinical isolates

The study was carried out between January 2003 and June 2004 in the three main public hospitals that serve nearly all the population of the island of Majorca, Spain: Hospital Universitari Son Dureta (HUSD; 800 beds), which is the tertiary reference hospital of the Balearic Islands; Hospital Son Llàtzer (330 beds); and Hospital de Manacor (200 beds). Only the first clinical isolate of MRSA (isolates from colonisation studies were not included) from each patient was included in the molecular epidemiology study, except for 18 patients who were admitted to two different hospitals at different times, for whom the first isolate from each admission was included. Types of clinical sample and hospital wards were recorded for MRSA-positive patients. Isolates were identified as *S. aureus* by standard microbiological procedures [14].

Antimicrobial susceptibility testing

Susceptibilities to oxacillin, vancomycin, teicoplanin, ciprofloxacin, erythromycin, clindamycin, gentamicin, mupirocin, fusidic acid, rifampicin and trimethoprim–sulphamethoxazole were determined by disk-diffusion according to CLSI recommendations [15]. The breakpoints used for fusidic acid (susceptible (S) ≥28 mm, intermediately-susceptible (I) 24–27 mm, resistant (R) ≤23 mm) and mupirocin (S ≥14 mm, R ≤13 mm) were those recommended by the disk manufacturer (Rosco Diagnostica, Taastrup, Denmark). The classical phenotypes of macrolide–lincosamide–streptogramin B (MLS_B) resistance were defined as follows: constitutive (c)MLS_B, erythromycin- and clindamycin-resistant; inducible (i)MLS_B, erythromycin-resistant, with clindamycin resistance inducible by erythromycin (presence of antagonism between the two disks); M phenotype, erythromycin-resistant and clindamycin-susceptible (absence of antagonism).

Molecular epidemiology studies

All MRSA isolates were characterised by macrorestriction analysis of chromosomal DNA after *Sma*I digestion and separation of the fragments by pulsed-field gel electrophoresis (PFGE) using a CHEF-DR III contour-clamped homogeneous electric field apparatus (Bio-Rad Laboratories, Richmond, CA, USA), programmed at 200 V (6 V/cm) for 23 h, with switching times ramped from 1 to 3 s. DNA fragments were visualised by staining with ethidium bromide and photographed under UV illumination, and were then interpreted following criteria recommended previously [16]. Control MRSA strains comprised: the three major clones (designated A, B and C) detected in previous studies at HUSD [17]; the two predominant MRSA clones, designated P_1 and Q_1, found in a multicentre Spanish study in 2004 [12]; the pandemic Iberian clone (ATCC BAA-44) [18]; the Brazilian clone (ATCC BAA-43) [19]; the Hungarian clone (ATCC BAA-39) [20]; the New York–Japan clone (ATCC BAA-41) [21]; the paediatric clone (ATCC BAA-42) [22]; and two isolates of EMRSA-15 (DEN4561 and HAR22) [23,24]. The reference strain *S. aureus* NCTC 8325 [16] was used as a molecular size standard to normalise the PFGE profiles. The nomenclature for MRSA clones in the present study was based on that established for previous collections [17].

Data analysis

Statistical analysis of the categorical variables was performed using Fisher's exact test and SPSS software (SPSS Inc., Chicago, IL, USA), with $p < 0.05$ considered to be statistically significant.

RESULTS

During the period of the study, 389 sequential MRSA isolates (202 from HUSD, 154 from Hospital Son Llàtzer, 33 from Hospital de Manacor) were recovered from 371 patients in the three participating hospitals, representing 31% of the patients with *S. aureus*-positive cultures. For 170 (46%) of the 371 patients, MRSA was isolated from only one sample type, whereas it was isolated from two or more sample types for 201 (54%) patients. The most frequent source of MRSA was exudates, mainly wound infections, ulcers and abscesses (49% of the patients), followed by the respiratory tract (37%). MRSA-positive blood cultures and intravenous catheters were documented for 14% and 7% of the patients, respectively.

Analysis of the PFGE patterns of the 389 MRSA isolates revealed that 366 (94%) belonged to three main clonal types (A, B and C) (Fig. 1). The remaining 23 (6%) MRSA isolates had different PFGE patterns, each being detected in <1.5% of the patients, and were therefore considered to be sporadic clones. Clone A accounted for 132 (34%) isolates, clone B for 109 (28%) isolates, and clone

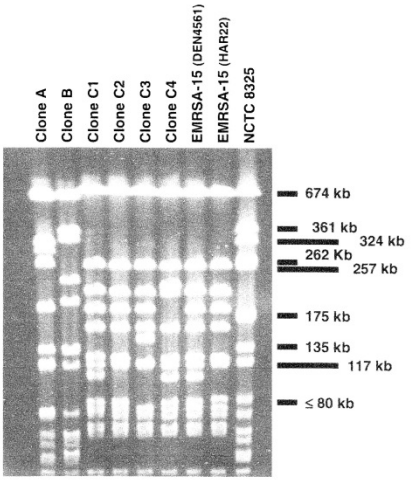

Fig. 1. *Sma*I macrorestriction patterns of clone A, clone B and the four subtypes of clone C (C1–C4). Two isolates of EMRSA-15 are included for comparison. Reference strain NCTC 8325 was used as the molecular size standard.

Table 1. Distribution of the major methicillin-resistant *Staphylococcus aureus* (MRSA) clones, grouped according to the hospital, hospital department and source of isolation

	Total MRSA *n* (%)	Clone A *n* (%)	Clone B *n* (%)	Clone C *n* (%)	Others *n* (%)
Overall	389 (100)	132 (34)	109 (28)	125 (32)	23 (6)
Hospital					
HUSD	202 (52)	63 (31)	70 (35)	57 (28)	12 (6)
Son Llàtzer	154 (40)	64 (42)	34 (22)	51 (33)	5 (3)
Manacor	33 (8)	5 (15)	5 (15)	17 (52)	6 (18)
Department[a]					
Medical wards	182 (46)	69 (38)	42 (23)	61 (33)	10 (6)
Surgical wards	75 (19)	22 (29)	30 (40)	17 (23)	6 (8)
ICU	43 (11)	14 (32)	18 (42)	11 (26)	0 (0)
Non-hospitalised	66 (16)	17 (26)	14 (21)	29 (44)	6 (9)
Source of isolation[a]					
Blood	51 (13)	13 (25)	8 (15)	27 (52)	3 (5)
Respiratory tract	137 (35)	53 (38)	43 (31)	36 (26)	4 (2)
Exudates	190 (48)	61 (32)	53 (27)	64 (33)	12 (6)

[a]Data for isolates from other or unknown departments (n = 23) and sources of isolation (n = 11) not shown.
HUSD, Hospital Universitari Son Dureta; ICU, intensive care unit.

C for 125 (32%) isolates (Table 1). These major clones were not related to any of the five previously described pandemic clones, including the Iberian clone, but clones A and B have been shown previously to be related to the two most prevalent clones (clones P_1 and Q_1) found in Spain at the present time. Clone C had a PFGE pattern identical to that of the EMRSA-15 epidemic clone (Fig. 1). Four subtypes of this clone (C1–C4) were detected (Fig. 1); the C1 subtype showed a PFGE pattern identical to that of one of the known EMRSA-15 control isolates (DEN4561), and the C2 subtype was identical to the other control isolate (HAR22).

All three major clones were detected in all three participating hospitals, although with slight differences among the different institutions (Table 1). In HUSD, the most frequent clone was clone B, isolated from 35% of patients with MRSA-positive cultures (p 0.002 compared with the other hospitals, 21%). In contrast, clone A, detected in 42% of patients, was the most frequent clone in Hospital Son Llàtzer (p <0.001 compared with the other hospitals, 29%), and clone C (52%) was the most frequent clone in Hospital Manacor (p <0.001 compared with the other hospitals, 30%).

Several interesting differences were observed when the distributions of MRSA clones from patients on different wards were compared (Table 1). The prevalence of MRSA clone B was significantly higher among patients admitted to intensive care units (ICUs) and surgical wards, being isolated from 42% of all patients with MRSA-positive cultures in ICUs, compared with 26% in non-ICU wards (p 0.003), and from 40% of all patients with MRSA-positive cultures in surgical units (p <0.001). No significant differences were observed in terms of the distribution of clone B in the ICUs of the three participating hospitals, although most (93%) MRSA isolates from ICUs were recovered from just two hospitals. Moreover, the overall high prevalence (47%) of clone B among patients in surgical wards was caused largely by the contribution from a single hospital.

In contrast, clone C (EMRSA-15) was isolated more frequently from non-hospitalised patients (emergency room and outpatient departments), being isolated from 44% of patients with MRSA-positive cultures in these departments, compared with 30% of hospitalised patients (p <0.001), suggesting that this clone is widespread in the community.

Interesting differences were also observed when the different sources of infection were compared (Table 1). Remarkably, the proportion of clone C (EMRSA-15) among blood specimens (52%) was significantly higher (p <0.001) than among other specimens (29%). Moreover, positive

Table 2. Antibiotic resistance rates among 389 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from Majorca, Spain

Antibiotic	Total MRSA (n = 389) n (%)	Clone A (n = 132) n (%)	Clone B (n = 109) n (%)	Clone C (n = 125) n (%)	Others (n = 23) n (%)
Ciprofloxacin	373 (96)	128 (97)	109 (100)	122 (98)	14 (61)
Gentamicin	*128 (33)*	*17 (13)*	*101 (93)*	*6 (5)*	*4 (17)*
Trimethoprim–sulphamethoxazole	4 (1%)	0	3 (3%)	0	1 (4%)
Vancomycin	0	0	0	0	0
Teicoplanin	0	0	0	0	0
Rifampicin	9 (2)	5 (4)	3 (3)	1 (1)	0
Mupirocin	21 (5)	10 (8)	11 (10)	0	0
Fusidic acid	16 (4)	6 (4)	2 (2)	7 (6)	1 (4)
Erythromycin	272 (70)	83 (63)	104 (95)	75 (60)	10 (43)
Clindamycin					
$cMLS_B$	212 (55)	78 (59)	100 (92)	25 (20)	9 (39)
$iMLS_B$	60 (15)	5 (4)	4 (4)	50 (40)	1 (4)

$cMLS_B$, constitutive macrolide–licosamide–streptogramin B (MLS_B) resistance; $iMLS_B$, inducible MLS_B resistance.

blood cultures were documented for 21% of the patients who were positive for this clone, compared with 9% of patients positive for other clones (p 0.001). The percentages of patients with positive blood cultures who were infected by the two other major clones (A or B) were only 10% and 7%, respectively.

Table 2 shows the antimicrobial resistance rates among all 389 MRSA isolates, as well as for the different clonal types. Overall, the MRSA isolates were uniformly susceptible to vancomycin and teicoplanin, and showed low rates of resistance to mupirocin (5%), fusidic acid (4%), rifampicin (2%) and trimethoprim–sulphamethoxole (1%). However, since disk-diffusion was used to test susceptibility, the presence of isolates with low-level glycopeptide resistance cannot be ruled out. Most (96%) isolates were resistant to ciprofloxacin, whereas resistance to erythromycin (70%), clindamycin (55% with $cMLS_B$; 15% with $iMLS_B$) and gentamicin (33%) was more variable. Clone B was associated with the highest rates of multiresistance, showing 100% resistance to ciprofloxacin (p 0.004 compared with other clones), 95% to erythromycin (p <0.001), 92% to clindamycin, plus an additional 3% with the $iMLS_B$ phenotype (p <0.001), and 93% to gentamicin (p <0.001). In contrast, clone C (EMRSA-15) showed the lowest rates of multiresistance among the three major clones, and a significantly lower level (5%) of resistance to gentamicin (p <0.001). Interestingly, clone C frequently showed the $iMLS_B$ phenotype (40% of all clone C isolates, including 67% of those resistant to erythromycin), whereas this phenotype was infrequent among the other clonal types (4% of all the non-clone C isolates, including 5% of those resistant to erythromycin) (p <0.001).

DISCUSSION

During the last four decades, methicillin resistance in *S. aureus* has been a problem of global dimensions, affecting mainly hospitalised patients, although MRSA has also emerged as a community-acquired pathogen in recent years. The prevalence of MRSA in Europe follows a north-to-south gradient, being lowest in Scandinavian countries (<2%) and highest in southern Europe, e.g., Greece, Italy, France, Spain and Portugal (30–60%) [25]. In Spain, the prevalence of MRSA has increased continuously since 1986 (1.5%), reaching 31% in 2002 [13]. Also, as in other countries, MRSA infections originating in the community are no longer infrequent, accounting for 18% of community-acquired *S. aureus* infections during 2002 [13].

Although the prevalence (31%) of MRSA revealed in Majorcan hospitals by this study is very similar to that reported in the rest of Spain, the molecular studies revealed remarkable differences concerning the epidemiology of this pathogen on the island. In addition to the two predominant MRSA clones found in Spanish hospitals, a high prevalence (32%) of the epidemic clone EMRSA-15 was found. Clone C, now classified as EMRSA-15, was already present in Majorca in 1999 (10% of MRSA-positive patients), but its prevalence has increased dramatically in recent years, so that it accounted for almost one-third of patients with MRSA who attended Majorcan hospitals in the present study. EMRSA-15 is the most frequent clone isolated in UK hospitals [26], and has also been detected in Germany [27], Finland, Sweden, Belgium [28], Portugal [29], Australia [30] and New Zealand [31]. This clone was reported for the first time in Spain at a teaching hospital in Tenerife (Canary Islands) [32], but the overall prevalence of EMRSA-15 in Spanish hospitals was found to be low (0.7%) in a large study involving >2000 MRSA isolates recovered between 1996 and 2002 from 110 Spanish hospitals [11].

The differential high prevalence of EMRSA-15 in Majorcan hospitals may be linked to the fact that the island is a frequent tourism destination (as is the Canary Islands) for individuals from countries with a high prevalence of this clone,

which perhaps highlights the importance of travel in the international spread of multiresistant pathogens. The prevalence of EMRSA-15 was even higher among non-hospitalised patients (44% vs. 30%), suggesting that, particularly for this clone, the flux was mainly from the community to the hospital setting, and not the other way around (as might be expected). The increase in MRSA infections in healthcare institutions other than hospitals could also contribute to this inverted flux. A study of patients with decubitus ulcers in a geriatric institution in Majorca between January 2000 and June 2002 revealed a very high prevalence of MRSA (70%), and the proportion of clone C (EMRSA-15) was already high (30%) during that period compared with 10% in the hospital setting [33].

A further interesting finding associated with EMRSA-15 is that, despite showing the lowest frequency of multidrug resistance among the three major clones, it was apparently more virulent, since it was associated significantly with bacteraemia. Positive blood cultures were documented for 21% of the patients infected by this clone, compared with <10% for the other major clones. Analysis of the interplay among pathogenicity, epidemicity and antibiotic resistance is a key element in understanding the evolution of microbial pathogens [34]. Several lines of evidence support the hypothesis of an inverse relationship between antibiotic resistance and epidemicity and pathogenicity in MRSA. Displacement of multidrug-resistant MRSA clones by more susceptible lineages has been described in recent years and, particularly for gentamicin resistance, has been found to be linked with increased fitness of the susceptible lineages and a reduction in the use of gentamicin [12,35–38]. The type of staphylococcal chromosomal cassette (SCC) *mec* complex could also play a role, since the SCC*mec* type showing the most efficient dissemination (SCC*mec*IV) is also associated with reduced antimicrobial resistance [39,40]. Both gentamicin susceptibility (as shown in this study) and the presence of SCC*mec*IV [5] are characteristics of EMRSA-15. Nevertheless, the possible association between EMRSA-15 and bacteraemia, and whether this is related to reduced antimicrobial resistance, needs to be confirmed in clinical studies designed with this specific aim. There are important clinical implications, in that bacteraemia caused by MRSA is associated with increased mortality compared with that caused by methicillin-susceptible strains [41].

In conclusion, widespread dissemination of the epidemic EMRSA-15 clone was found throughout Majorca, and this clearly differs from the situation documented in mainland Spanish hospitals. In addition, a statistically significant association was found between this clone and lower antimicrobial resistance rates, non-hospitalised patients and bacteraemia.

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Prevalence of methicillin-resistant *Staphylococcus aureus* and factors associated with colonization among residents in community long-term-care facilities in Spain

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ABSTRACT

Hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strains are no longer limited to acute-care hospitals but have now spread to other healthcare settings such as long-term-care facilities (LTCFs), in most of which they are endemic. In Europe, few studies have addressed the MRSA situation in LTCFs. A cross-sectional study to determine MRSA prevalence and factors associated with *S. aureus* carriage in community LTCF residents is reported here. Nasal and decubitus ulcer cultures were performed for residents of nine community LTCFs. Residents were classified as MRSA carriers, methicillin-susceptible *S. aureus* carriers and non-carriers. Overall, 1377 nasal swabs and 82 decubitus ulcer cultures were performed. MRSA was isolated from 15.5% and 59.0% of the former and latter, respectively. The prevalence of MRSA colonization was 16.8% (95% CI 14.9–18.8), varying from 6.7% to 35.8% ($p < 0.001$) among LTCFs. Several independent variables were related to MRSA colonization. It is noteworthy that residents in an LTCF with fewer than 150 beds had at least a two-fold higher probability of being MRSA carriers. Modifiable factors were medical devices, decubitus ulcers and previous antibiotic treatment. An age of 85 years or older, a Charlson index ≥2 and transfer from an acute-care facility were non-modifiable factors also related to MRSA colonization. A high MRSA prevalence among residents in community LTCFs in Spain, with great variability among facilities, was found. The factors identified as being associated with MRSA colonization could be prevented by the implementation of several measures. Control strategies need to be coordinated between LTCFs and acute-care hospitals.

Keywords Epidemiology, geriatrics, long-term care, methicillin-resistant *Staphylococcus aureus*, MRSA

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INTRODUCTION

The introduction and successful dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) in acute-care hospitals in Spain was reported two decades ago [1,2], and it has become the major problem related to multiresistant microorganisms in the healthcare system. Furthermore, MRSA strains have spread into facilities related to the healthcare setting, such as long-term-care facilities (LTCFs), and are endemic in the majority of them [3,4]. Control measures to limit MRSA spread in LTCFs are controversial [5]. Most studies on the prevalence of MRSA colonization in LTCFs have been performed in Veterans' Affairs facilities in the USA; however, the epidemiology of MRSA in community LTCFs should be differentiated from those facilities [6]. In Europe, some studies have

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recently evaluated the prevalence of MRSA colonization in these facilities [7–10]. In Spain, only a few studies have addressed the interaction of MRSA strains between hospitals and other healthcare facilities [11–14]. An interesting finding is that patients with *S. aureus* bloodstream infection diagnosed at hospital admission following referral from an LTCF were associated with the presence of methicillin resistance [14]. Patients in whom MRSA carriage acquired during a previous hospital admission or stay in a LTCF was persistent were found to be at high risk of MRSA infection, although a low prevalence of these infections has been reported in LTCF residents [15,16]. Transfer of patients from acute-care hospitals to LTCFs is often delayed because of MRSA carriage. To determine the extent of this situation, a cross-sectional study was conducted in a large number of residents in community LTCFs for the elderly. The aims were to determine the prevalence of MRSA colonization and to define factors associated with *S. aureus* carriage in this population.

MATERIALS AND METHODS

Study design

This was a cross-sectional prevalence survey.

Study population and characteristics of community LTCFs

The study was performed in November 2005, in residents of community LTCFs for the elderly, located in two communities in Spain (Catalonia and the Balearic Islands).

Nine LTCFs with a total of 1586 beds (median 120; range 72–552 beds) were included in the study. Five of these are allocated to the geographical area of influence of a 900-bed acute-care hospital (Hospital Universitari de Bellvitge), three to that of a 490-bed hospital (Corporació Sanitària Parc Taulí) and one to that of an 800-bed acute-care hospital (Hospital Universitari Son Dureta). These facilities provide care for elderly permanent residents who may be disabled or infirm. All of these facilities have their own medical services, and most include a dementia ward. Residents are accommodated in rooms with one, two or three beds.

These LTCFs do not use active surveillance cultures to detect MRSA carriage, and decolonization procedures are not routinely performed. All facilities take standard precautions for patient care, and contact precautionary measures are occasionally applied for those patients colonized or infected by MRSA. A common policy of all the facilities is that known MRSA carriers are not denied admission.

Data collected

Medical charts were reviewed for basic clinical and epidemiological information. The following data were obtained for all residents: age, sex, facility and date of admission, underlying diseases (Charlson index) [17], functional status (Barthel index) [18], prior MRSA isolation, presence of decubitus ulcers, previous antibiotic treatment in the last 3 months, use of invasive devices (peripheral venous catheter, nasogastric tube and urinary catheter) in the last 7 days, and the presence of current infections.

Microbiological methods

To assess MRSA colonization, samples for cultures were obtained from anterior nares and third-degree or higher-degree decubitus ulcers, when applicable. These areas were swabbed with sterile cotton-tipped applicator sticks, which were immediately placed into Stuart transport medium. Nasal and decubitus ulcer swabs were first plated onto coagulase–mannitol agar plates and selective MRSA agar medium (MRSA Select; Bio-Rad Laboratories, Madrid, Spain). Swabs were then inoculated into staphylococcal enrichment broth composed of brain–heart infusion plus 7% NaCl. After 24 h of incubation at 35°C, the broth was subcultured onto coagulase–mannitol and MRSA Select plates. All plates were incubated for 48 h and inspected daily. Suspected *S. aureus* colonies were identified by the latex agglutination test (Pastorex® Staph-plus; Bio-Rad Laboratories) and DNase production (DNase Test Agar; Biomedics, Madrid, Spain). Methicillin resistance was determined by the cefoxitin disk diffusion method following CLSI recommendations. Testing for antimicrobial susceptibility to penicillin, oxacillin, cefoxitin, erythromycin, clindamycin, gentamicin, tobramicin, rifampin, tetracycline, trimethoprim–sulphamethoxazole, chloramphenicol, ciprofloxacin, vancomycin, teicoplanin, mupirocine, fusidic acid, quinupristin–dalfopristin and linezolid was performed by the disk diffusion method. [19].

Statistical analysis

For the purpose of this study and on the basis of the results, residents were classified as MRSA carriers, methicillin-susceptible *S. aureus* (MSSA) carriers and non-carriers. Following the methodology suggested by Harris *et al.* [20], two separate analyses were performed to compare, first, MSSA carriers with non-carriers, and second, MRSA carriers with non-carriers. Results were interpreted as follows: significant variables in the former analysis were considered to represent unique factors associated with MSSA colonization, and those in the second were considered to be factors associated with MRSA colonization. Variables with statistical significance in both analyses were considered to be factors related to *S. aureus* carriage.

Categorical variables were analyzed with chi-square tests or Fisher's exact test, as appropriate. Continuous variables were analyzed with Student's *t*-test or non-parametric tests. Variables associated with MRSA carriage with a probability equal to or less than 0.1 were further examined using advanced multivariable logistic regression modelling. All statistical tests were two-tailed, and p-values <0.05 were considered to be significant. SPSS package version 12.0 was used.

Approval for the study was obtained from the Research Ethics Committee of the Hospital Universitari Bellvitge. No written informed consent was obtained, because the study met the criteria for a waiver of this requirement.

Table 1. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization and clinical characteristics of residents in different community long-term-care facilities included in the study

	Centres (number of beds)									
	1 (220)	2 (120)	3 (552)	4 (72)	5 (101)	6 (94)	7 (121)	8 (124)	9 (182)	Total (1586)
Residents, *n*	195	115	466	72	95	89	109	73	163	1377
Male sex (%)	35.4	21.7	31.8	18.1	22.1	15.7	39.4	26.0	25.8	28.6
Mean age (years)	76.1	82.9	82.2	83.9	83.2	83.9	77.2	83.0	81.3	81.2
Charlson index ≥2%	73.1	47.0	35.2	47.2	67.4	58.0	70.4	40.3	66.7	52.8
Barthel index <30%	55.4	23.5	22.2	27.8	44.2	40.9	75.2	19.4	14.2	33.4
Decubitus ulcers (%)	12.3	6.1	5.8	2.8	8.4	8.0	30.2	1.4	1.8	8.1
Invasive devices use (%)	9.2	2.6	1.7	1.4	3.2	2.3	27.6	5.6	0	5.0
Prior antibiotic treatment (%)	29.7	22.6	10.3	12.5	23.2	18.2	44.0	40.8	36.2	22.5
Prior MRSA carriage (%)	21.8	1.7	1.3	6.9	6.3	0	2.8	0	8.0	5.6
Stay ≥6 months (%)	72.4	92.2	91.8	93.1	90.4	93.0	60.2	54.9	94.4	85.1
MSSA colonization (%)	16.9	8.7	24.3	16.7	15.8	24.7	29.4	27.4	11.8	16.7
MRSA colonization (%)	22.6	24.3	8.4	15.3	28.4	6.7	35.8	27.4	10.4	16.8

MSSA, methicillin-susceptible *Staphylococcus aureus*.

RESULTS

Characteristics of patients

All residents present on the day of the study (1377) were included in the analyses. Characteristics of residents as ascribed by community LTCFs are shown in Table 1. Among them, 71.4% were women; the mean age was 81.2 years (SD 9.9 years). Underlying conditions were present in 1138 (82.6%) residents. The most frequent of these conditions were dementia in 548 (39.8%) residents, diabetes mellitus in 321 (23.3%), chronic obstructive pulmonary disease in 206 (15%), solid tumours in 194 (14.1%), and hemiplegia in 169 (12.3%). The median Charlson index was 2 (interquartile range: 1–3) points, and the mean Barthel index was 54.16 (SD 38.3) points.

Prevalence of MRSA colonization

Cultures were performed from a total of 1377 nasal swabs and 82 decubitus ulcer swabs. *S. aureus* carriage was observed in 33.5% of the residents; 50.0% showed methicillin resistance. Overall, 231 (16.8%) residents were colonized by MRSA, 230 (16.7%) were colonized by MSSA, and the remaining 916 (66.5%) were non-carriers. The prevalence of MRSA colonization among residents was 16.8% (95% CI 14.9–18.8), varying from 6.7% to 35.8% ($p < 0.001$) among the LTCFs (Table 1). MRSA was isolated from 213 (15.5%) nasal swabs and from 49 (59.0%) decubitus ulcers. Eighteen residents with decubitus ulcers colonized by MRSA had a concomitant negative nasal swab, and in 31 residents both sites were colonized. Prior MRSA carriage was known in 77 (33.3% of the residents colonized by MRSA). No MRSA infection was recorded during the cross-sectional study.

Factors associated with *S. aureus* colonization in community LTCF residents

Table 2 shows bivariate and multivariate analysis comparing MRSA carriers with non-MRSA carriers. MSSA carriers had a higher Charlson index, more often had invasive devices, and more often had been transferred from hospitals and resided in facilities with <150 beds, as compared with non-carriers (data not shown). Variables associated with MRSA colonization were age of 85 years or more, existence of decubitus ulcers, previous antibiotic treatment, presence of invasive devices, comorbidity, transfer from an acute-care hospital, and residence in facilities with <150 beds. Multivariate analysis to identify independent factors associated with MSSA and MRSA colonization are shown in Tables 3 and 4, respectively. A comparison of the two models showed that for MSSA carriers, a Charlson index ≥2 points and residence in a facility with <150 beds remained independent factors. On the other hand, several independent variables were related to MRSA colonization. It is noteworthy that residents of a facility with <150 beds had at least a two-fold higher probability of being MRSA carriers (OR 2.10; 95% CI 1.52–2.92). Other modifiable factors were medical devices, presence of decubitus ulcers, and previous antibiotic treatment. Age of 85 years or more, a Charlson index ≥2 points and transfer from an acute-care hospital were non-modifiable factors also related to MRSA colonization.

	MRSA carriers, n = 231 n (%)	Non-MRSA carriers, n = 1146 n (%)	Univariate OR MRSA/non-MRSA carriers (95% CI)	Multivariate OR MRSA/non-MRSA carriers (95% CI)
Sex (male)	72 (31.2)	322 (28.1)	1.15 (0.85–1.57)	
Age ≥85 years	95 (41.3)	380 (33.4)	1.41 (1.05–1.88)	1.60 (1.16–2.21)
Charlson index ≥2 [17]	148 (64.6)	567 (50.4)	1.79 (1.33–2.41)	1.50 (1.09–2.08)
Barthel index <30 [18]	98 (43.0)	344 (31.4)	1.65 (1.23–2.21)	1.02 (0.72–1.43)
Decubitus ulcers	46 (20.1)	65 (5.7)	4.17 (2.77–6.28)	2.56 (1.58–4.17)
Previous antibiotic	97 (42.9)	207 (18.4)	3.33 (2.13–4.42)	2.44 (1.75–3.39)
Medical devices	31 (13.5)	37 (3.2)	4.67 (2.83–7.71)	2.47 (1.35–4.52)
Transferral from acute-care centre	55 (24.1)	103 (9.4)	3.07 (2.13–4.42)	2.15 (1.39–3.31)
Stay ≥6 months	176 (77.9)	943 (86.6)	0.54 (0.38–0.78)	0.80 (0.53–1.22)
Centre with <150 beds	131 (56.7)	422 (36.8)	2.25 (1.69–2.99)	1.77 (1.29–2.41)

Comparison of patients colonized by MRSA with a control group of non-MRSA carriers.

Table 2. Bivariate and multivariate analysis of associated factors for methicillin-resistant *Staphylococcus aureus* (MRSA) colonization

Table 3. *Associated factors for methicillin-susceptible Staphylococcus aureus* carriage vs. no *S. aureus* carriage

	OR	95% CI	p
Age ≥85 years	0.84	0.61–1.17	0.31
Charlson index ≥2	1.95	1.31–2.90	0.001
Barthel index <30	1.07	0.76–1.50	0.70
Decubitus ulcers	1.24	0.65–2.36	0.51
Previous antibiotic use	0.77	0.52–1.15	0.20
Medical devices	1.50	0.66–3.39	0.33
Transferral from acute-care centre	1.45	0.88–2.40	0.14
Stay ≥6 months	0.73	0.48–1.12	0.16
Centre with <150 beds	1.69	1.24–2.31	0.001

Logistic regression model.

Table 4. Associated factors for methicillin-resistant *Staphylococcus aureus* carriage vs. no *S. aureus* carriage

	OR	95% CI	p
Age ≥85 years	1.56	1.13–2.19	0.009
Charlson index ≥2	2.36	1.57–3.55	<0.001
Barthel index <30	1.08	0.76–1.55	0.64
Decubitus ulcers	2.92	1.73–4.93	<0.001
Previous antibiotic	2.20	1.56–3.13	<0.001
Medical devices	3.05	1.56–5.97	0.001
Transferral from acute-care centre	2.52	1.59–4.02	<0.001
Stay ≥6 months	0.80	0.51–1.26	0.34
Centre with <150 beds	2.10	1.52–2.92	<0.001

Logistic regression model.

DISCUSSION

In Europe, there is little information on the prevalence of MRSA colonization among LTCF residents. Recent studies have reported prevalences of 22% in the UK [7], 9.3% in Slovenia [8], 8.6% in Ireland [10] and 1.1% in Germany [9]. These figures are lower than those reported in studies of Veterans' Affairs facilities in the USA, where the colonization rate can reach 45% [6,21]. To our knowledge, this is the first report on the prevalence of MRSA colonization in community LTCFs in Spain.

In a previous study, the clinical epidemiology of patients with *S. aureus* bloodstream infections upon hospital admission was analyzed, and the major risk factors for methicillin resistance were prior MRSA isolation and having been transferred from an LTCF [14]. Given the increasing rate of exchange of patients between facilities in the healthcare setting and acute-care hospitals, it seems pertinent to determine the magnitude of MRSA colonization in LTCFs, as the extent of this problem could influence the infection control practices implemented by hospitals [22]. In Spain, guidelines for the prevention of MRSA transmission in acute-care hospitals are well established [23], even though there is a lack of such recommendations for LTCFs. The policy of most facilities is to admit MRSA carriers, although transfer of these patients from acute-care centres is often limited. This is because healthcare personnel have not been instructed on the measures for handling MRSA-colonized patients, and contact precaution measures are costly.

We analyzed a large number of patients from a homogeneous type of LTCF, i.e. community LTCFs, and performed cultures from nasal swabs and decubitus ulcers, which has been reported to be a valid and efficient method for the detection of MRSA carriage [24,25]. It is worthy of note that 18 residents in this study had decubitus ulcers colonized by MRSA and a concomitant negative nasal swab. No prior decolonization was recorded in their medical records, and intestinal MRSA carriage was not ruled out [26]. A high prevalence of MRSA colonization was found among patients within these institutions. This study allows an appreciation with further detail of the MRSA situation in the healthcare system. However, it is important to emphasize that active surveillance within LTCFs is not recommended in the absence of surveillance of suspected outbreaks of infection; thus, this study is not reproducible in daily clinical practice. In LTCFs, clinical samples and medical

records from hospitals should be considered, to define the baseline rate of MRSA colonization within a facility [21]. In the present study, approximately one-third of the residents colonized by MRSA were previously recognized as MRSA carriers by the facility; this indicates irregular application of the aforementioned recommendations. An outstanding finding is the wide variation in the prevalence of MRSA colonization among facilities, something that has been pointed out in other reports [21]. Differences in colonization rates may depend on several factors, such as the prevalence of MRSA in the referral hospital and infection control practices at the LTCF.

To determine factors associated with MRSA colonization, two separate models using multivariate analysis were built. This is an effective method for identifying factors associated with resistant pathogens, particularly for studies on infection control [20,27]. In this analysis, only the size of facilities was considered as a specific factor related to MRSA colonization; thus, it is difficult to interpret the differences found in the prevalence of MRSA colonization among LTCFs. A limitation of this point-prevalence study is the absence of a second sample to confirm the residents' carrier status; consequently, it was not possible to detect intermittent MRSA carriage. In LTCFs, the rate of intermittent carriage of MRSA is approximately 15% [26]. The study design makes it difficult to determine the exact risk of MRSA colonization; therefore, only associated factors are referred to. Independent factors associated with *S. aureus* carriage in community LTCF residents were comorbidity and the size of the facilities. Independent factors associated with MRSA colonization among this population were presence of decubitus ulcers, previous antibiotic treatment, age of 85 years or more, use of invasive devices, and transfer from an acute-care hospital. Limited data are available concerning risk factors for MRSA colonization in LTCFs in Europe. Vovko *et al.* [28] found previous antibiotic treatment and hospital admission to be risk factors in an LTCF with a low number of MRSA carriers. Interestingly, in other studies, care facility-specific risk factors for MRSA colonization, e.g. low ratio of nurses to beds, have been associated with MRSA carriage [7,29]. In our model, there is no variable that could explain why centres with <150 beds constitute an independent factor associated with *S. aureus* colonization, but we have observed that some of these facilities are deficient in application of standard measures to limit cross-transmission. A stay of more than 6 months in an LTCF has been found to be associated with the rate of MRSA colonization [29], in contrast to the findings of other authors [9,10]. In the present study, a stay of fewer than 6 months in an LTCF was associated with MRSA colonization only in the bivariate analysis; this should be interpreted as a survival bias due to the study design.

These observations show that factors related to methicillin resistance among residents in community LTCFs are similar to those described in hospitalized patients [30]. It is noteworthy that modifiable factors could be prevented by the introduction of only a few infection control measures. Appropriate, individualized and easy-to-implement infection control precautions, together with the promotion of a healthy lifestyle for residents, should be observed in LTCFs. Hand washing (alcohol-based gel) protocols, educational programmes for healthcare personnel, guidelines for antibiotic use and enhanced efforts to prevent the development of decubitus ulcers would all be suitable measures for community LTCFs.

To conclude, this study reports a large reservoir of MRSA among community LTCFs in Spain. The results indicate that restriction of referral of MRSA carriers from acute-care hospitals to community LTCFs would not be effective, because there is a high prevalence of silent carriers. Patients transferred from LTCFs to hospitals should be considered at high risk for MRSA colonization and should be included in a screening programme at admission. We therefore recommend that control strategies be coordinated between LTCFs and acute-care hospitals. Further studies are necessary to define the clinical impact of MRSA colonization among residents in community LTCFs.

TRANSPARENCY DECLARATION

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cuenta que ambos métodos utilizan anticuerpos de una misma procedencia. Aunque la confirmación definitiva de algunos serotipos precisa ser efectuada mediante el test de Quellung, nuestros resultados coinciden con los de otros autores[2] y apuntan a que el empleo de Pneumotest-Latex resulta muy útil como método inicial de tipificación. Si bien no debe considerarse una alternativa excluyente respecto al test de Quellung, dada su comodidad y accesibilidad a personal menos entrenado y su capacidad para la correcta identificación de serogrupos, su uso puede reducir enormemente el número de determinaciones que realizar por esta técnica de referencia. Entre sus limitaciones hay que destacar, además de la falta de poder discriminativo dentro de determinados serogrupos, su elevado precio.

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Neumonía nosocomial por *Staphylococcus aureus* resistente a meticilina de origen comunitario productor de leucocidina de Panton-Valentine

Sr. Editor: La leucocidina de Panton-Valentine (LPV), identificada en 1932[1], es una exotoxina específica de *Staphylococcus aureus* con propiedades leucotóxicas en polimorfonucleares y macrófagos humanos. Está producida por menos del 1% de los aislados de *S. aureus*[2]. Las cepas de *S. aureus* productoras de LPV están relacionadas con infecciones piógenas de la piel (como los forúnculos) y, con menor frecuencia, con la neumonía necrosante grave[3]. También se ha asociado con el recientemente conocido *S. aureus* resistente a meticilina (SARM) comunitario[4]. A continuación presentamos el caso de un paciente con una neumonía nosocomial por SARM productor de LPV.

Varón de 34 años, natural de la India aunque residente en España desde hace 6 años, trabajador en la hostelería en Mallorca, fumador y bebedor importante (con dependencia alcohólica). Fue traído a urgencias en abril de 2007 por un traumatismo craneoencefálico con hematoma subdural. El paciente ingresó en la unidad de cuidados intensivos (UCI) intubado y conectado a ventilación mecánica, y recibió tratamiento antibiótico empírico con amoxicilina-ácido clavulánico. La cifra de leucocitos en sangre era de $12{,}05 \times 10^3/\mu l$. En el primer día del ingreso se realizó un broncoaspirado (BAS). En el cultivo creció una flora mixta respiratoria. A los 8 días del ingreso presentó fiebre y secreciones purulentas espesas, y en la radiografía de tórax se objetivó una neumonía en el lóbulo inferior izquierdo complicada con atelectasias bibasales. La concentración de leucocitos en sangre era en ese momento de $4{,}10 \times 10^3/\mu l$. Se recogió una nueva muestra de BAS en la que se aislaron más de 10^6 unidades formadoras de colonia (ufc) de *S. aureus*. En el antibiograma, realizado con el método de difusión con discos siguiendo los criterios del Clinical and Laboratory Standards Institute (CLSI), este aislado (cepa 05015862) era únicamente resistente a todos los antibióticos betalactámicos (incluyendo la oxacilina), y sensible a la eritromicina, clindamicina, ciprofloxacino, cotrimoxazol, vancomicina, teicoplanina, rifampicina, linezolid, gentamicina, tobramicina, tetraciclina, cloranfenicol, mupirocina y ácido fusídico. Se suspendió el tratamiento antibiótico anterior y se administró vancomicina. En el posterior estudio de colonización de SARM (cultivo de exudados nasal, axilar e inguinal), realizado a los 10 días del ingreso, se aisló este microorganismo en el exudado nasal con el mismo patrón de resistencia. El paciente recibió tratamiento con ácido fusídico intranasal y lavados con clorhexidina en axilas e ingles durante 5 días. A los 15 días del ingreso se volvió a aislar SARM en el cultivo del BAS con el mismo antibiograma. A los 17 días se le retiró la intubación. La cifra de leucocitos a partir de este momento y hasta el alta se mantuvo entre 9 y $13 \times 10^3/\mu l$. A los 22 días, se aisló en el BAS más de 10^6 ufc de *Pseudomonas aeruginosa* sin aislarse SARM, por lo que se asoció ceftazidima y tobramicina a la vancomicina, y se administraron los tres antibióticos durante 18 días. A los 30 días, el paciente ingresó en planta. En un estudio de colonización, realizado a los 35 días, se aisló SARM en el exudado axilar con el mismo antibiograma, por lo que se repitieron los lavados con clorhexidina en axilas e ingles durante 5 días. Los siguientes tres estudios de colonización fueron negativos para SARM, la evolución fue buena y el paciente fue dado de alta y derivado a un centro de rehabilitación.

Se realizó una reacción en cadena de la polimerasa (PCR) múltiple en las dos primeras cepas de SARM del paciente (la del BAS y la del exudado nasal) para los genes *mecA* (resistencia a la meticilina), *nuc* (específico de *S. aureus*) y *luk-PV* (producción de LPV)[3]. En ambas cepas, la PCR fue positiva a los tres genes. Se utilizó como control positivo la cepa de SARM comunitario 3922-04, aislada en un absceso glúteo de un niño de origen ecuatoriano y proporcionada por la doctora Emilia Cercenado, del Hospital Gregorio Marañón de Madrid[5]. Se efectuó otra PCR múltiple para la tipificación del complejo del gen de la recombinasa del casete cromosómico (*ccr*)[6], en la que presentaron el casete cromosómico estafilocócico *mec*

(SCC*mec*) de tipo IV. En el estudio de epidemiología molecular por electroforesis en campo pulsado (ECP), usando *Sma*I como enzima de restricción, las dos cepas del paciente fueron idénticas entre sí, diferentes de los tres clones intrahospitalarios mayoritarios en Mallorca (clones A, B, C)[7], e interesantemente presentaron un patrón de bandas idéntico al de la cepa de referencia 3922-04 (fig. 1).

La neumonía producida por cepas de *S. aureus* productoras de LPV se caracteriza por ser más frecuente en adultos jóvenes previamente sanos y se suele manifestar con fiebre alta, hemoptisis, elevada frecuencia cardíaca y leucocitopenia, con una elevada mortalidad[8]. Recientemente, se están detectando en nuestro país algunas neumonías causadas por cepas de SARM productoras de LPV en localizaciones geográficas diversas como Tenerife[9] (clon ST125-MRSA-IV) y Galicia[10] (clon ST80-MRSA-IV), aunque, actualmente, el genotipo predominante en España entre las cepas de SARM productoras de LPV es el ST8-MRSA-IV, relacionado con el clon USA300, que es la cepa de SARM comunitaria más prevalente en los Estados Unidos[11].

El patrón de ECP de nuestra cepa, así como el de la cepa 3922-04, es idéntico al del clon (D1) descrito por primera vez en Madrid en 2003, responsable de varias infecciones cutáneas de origen extrahospitalario, particularmente en la población pediátrica[12]. Aunque no se ha realizado tipificación mediante *multilocus sequence typing* (MLST) de nuestra cepa, este clon parece pertenecer al ST8-MRSA-IV (Fernando Chaves, comunicación personal).

A pesar de ser la neumonía de adquisición nosocomial, lo más probable es que el paciente estuviera previamente colonizado por esta cepa en la orofaringe, puesto que la colonización por SARM puede durar de meses a años. Posteriormente, el paciente, tras la intubación y el tratamiento antibiótico con amoxicilina-ácido clavulánico como factores predisponentes, desarrollaría la neumonía por la aspiración de las secreciones orofaríngeas. Un estudio estima que hasta en el 80% de los casos globales de infección nosocomial por *S. aureus*, el paciente estaba colonizado por este microorganismo antes del ingreso[13]. Además, hasta el momento, esta cepa no se ha detectado en ningún otro paciente ingresado en nuestro hospital, incluyendo un estudio de colonización de SARM efectuado en los pacientes ingresados en la misma unidad de UCI, lo que va más a favor de la adquisición extrahospitalaria de dicha cepa. En cualquier caso, la emergente diseminación de clones de SARM productores de LPV en España obliga a mantener una vigilancia activa de este fenómeno por sus notables consecuencias clínicas y terapéuticas.

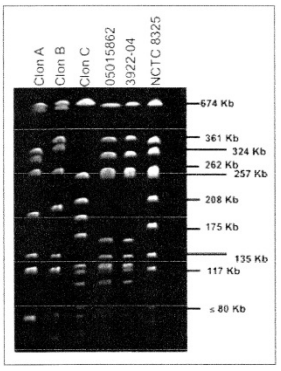

Figura 1. Electroforesis en campo pulsado de cepas de SARM usando *Sma*I como enzima de restricción. Carriles: 1 Clon A, 2 Clon B, 3 Clon C (EMRSA-15), 4 cepa 05015862, 5 cepa 3922-04 productora de LPV (proporcionada por la doctora Cercenado), 6 cepa control de *Staphylococcus aureus* NCTC 8325 utilizada como marcador de peso molecular.

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Staphylococcus aureus resistente a la meticilina de origen comunitario

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Recientemente, las cepas de *Staphylococcus aureus* resistente a la meticilina (SARM) han aparecido como una causa de infecciones adquiridas en la comunidad (CO) en pacientes sin los factores de riesgo establecidos para la infección por dichos patógenos. En niños y pacientes jóvenes previamente sanos producen, principalmente, infecciones leves de piel y partes blandas, pero también pueden causar fascitis y neumonía necrosante grave. Las cepas de SARM-CO se diferencian de las hospitalarias en su sensibilidad a múltiples clases de antimicrobianos y en sus características genéticas. La mayoría de ellas comparten el *cassette* cromosómico estafilocócico (SCC*mec*) de tipo IV y producen la leucocidina de Panton-Valentine (LPV), una citotoxina que provoca destrucción de los leucocitos y necrosis tisular. Actualmente, el clon predominante es el USA300, que se ha diseminado por Estados Unidos, Europa y Australia. En España, el clon predominante está relacionado con el USA300. El principal mecanismo de transmisión es por contacto directo entre personas, aunque también se han implicado en la transmisión algunos animales domésticos y de granjas. En los pacientes con infecciones purulentas de piel y partes blandas, la incisión y el drenaje constituyen el tratamiento fundamental, que se debe asociar a la administración de antibióticos si la respuesta es inadecuada. Se puede administrar clindamicina, cotrimoxazol o tetraciclinas y se debe evitar la utilización de fluoroquinolonas por su facilidad para desarrollar resistencia. En el caso de infecciones graves, la vancomicina sigue siendo el tratamiento de elección, si bien hay otras alternativas como el linezolid y la daptomicina (excepto en las neumonías). Las normas de higiene general son la medida más eficaz para evitar su diseminación.

***Palabras clave:* *Staphylococcus aureus* resistente a meticilina (SARM). Infecciones en la comunidad. SARM-CO. Leucocidina de Panton-Valentine.**

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Community-acquired methicillin-resistant *Staphylococcus aureus*

Recently, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a cause of community-acquired (CA) infections among patients without established risk factors for MRSA. CA-MRSA strains mainly cause mild skin and soft tissue infections in otherwise healthy children and young adults, but can also cause severe necrotizing fasciitis and pneumonia. In contrast to nosocomial MRSA, CA-MRSA are, in general, susceptible to multiple antimicrobials and present a different genotype. Most CA-MRSA strains share the staphylococcal chromosomal cassette (SCC*mec*) type IV and produce Panton-Valentine leukocidin (PVL), a cytotoxin that causes leukocyte destruction and tissue necrosis.
At present, the predominant clone is the USA300 clone, which is widely disseminated in the United States, Europe and Australia. In Spain, the predominant clone is related to the USA300 clone. The main mechanism of transmission is close person-to-person contact, although household pets and farm animals have also been implicated.
In patients with purulent skin and soft tissue infections, the mainstay of treatment is incision and drainage. Antimicrobials are indicated in patients not responding to appropriate drainage. Clindamycin, trimethoprim-sulfamethoxazole or tetracyclines can be administered, while the use of fluoroquinolones should be avoided due to the rapid emergence of resistance. For severe infections, vancomycin should be used. Other alternatives are linezolid or daptomycin (only if there is no pulmonary involvement). Adequate hygiene practices are the most efficient measure to prevent spread.

***Key words:* Methicillin-resistant *Staphylococcus aureus* (MRSA). Community-acquired infections. CA-MRSA. Panton-Valentine leukocidin.**

Introducción

Las cepas de *Staphylococcus aureus* con resistencia a la meticilina (SARM) son una de las principales causas de infecciones hospitalarias y, en general, de infecciones asociadas a los cuidados sanitarios (ACS)[1]. A pesar del aumento de la prevalencia de SARM en los hospitales, es-

tas cepas se han aislado con poca frecuencia en la comunidad. En muchas situaciones los SARM se detectan en la comunidad cuando los pacientes, todavía infectados o colonizados por estos microorganismos, son dados de alta de un centro hospitalario, o bien cuando se transmiten a la comunidad a partir de personal hospitalario colonizado. Estas cepas se asocian con infecciones que comienzan en la comunidad pero que presentan factores de riesgo hospitalarios. Sin embargo, en la última década, numerosas comunicaciones han descrito la existencia de infecciones por SARM en la comunidad, cuyo origen no está relacionado con el hospital o con los cuidados sanitarios[2,3]. Además, hay evidencia molecular que permite identificar a estas cepas de SARM como verdaderas comunitarias y que demuestra que también han evolucionado en la comunidad, se han adaptado bien a sobrevivir allí y están causando brotes comunitarios[4,5]. Estas cepas son las denominadas SARM de origen comunitario (SARM-CO) que producen infecciones en pacientes que no presentan los factores de riesgo establecidos para la infección por SARM: por ejemplo, no tienen una historia previa de infección o colonización por SARM; no han sido hospitalizados en el año previo al aislamiento ni han recibido asistencia en centros sociosanitarios ni de diálisis; no han sido sometidos a cirugía o implantación de catéteres permanentes u otros dispositivos médicos, y no son familiares de trabajadores sanitarios[6,7]. No obstante, una definición de SARM-CO que incluya únicamente los SARM aislados de personas sin factores de riesgo de adquisición hospitalaria puede excluir a pacientes infectados con cepas que genéticamente son SARM-CO, por lo que también se debe tener en cuenta las características genéticas de los aislados para definirlos como comunitarios[8]. Actualmente, el rápido aumento de la prevalencia de SARM-CO en algunos países, así como su reciente introducción y diseminación en los hospitales, ha difuminado las fronteras entre cepas de adquisición comunitaria y hospitalaria, y ha creado la necesidad de realizar una detección precoz y de reconsiderar nuevas estrategias terapéuticas[8,9].

Epidemiología

En los últimos 10 años la epidemiología de SARM ha cambiado. Los SARM-CO difieren de los hospitalarios en el espectro de la enfermedad y en la epidemiología. Estas cepas parecen tener un reservorio fuera del hospital, son causa principalmente de infecciones de piel y partes blandas, generalmente forúnculos y abscesos y, en ocasiones, de neumonía necrosante grave en niños y adultos jóvenes y sanos, y suelen producir pequeños brotes que se han descrito en determinados grupos de población, como en los aborígenes australianos, indios americanos, nativos de Alaska, reclusos, soldados en cuarteles, homosexuales, usuarios de drogas por vía parenteral, tatuados, equipos de deportistas (principalmente de deportes de contacto) y guarderías[9-11]. Se ha indicado que la exposición previa a antimicrobianos puede ser un factor de riesgo para la adquisición de SARM-CO, aunque no con tanta frecuencia como ocurre en los aislados nosocomiales[6], y que estas cepas también se pueden transmitir a partir de animales de compañía[12] y de algunos animales de granja (caballos y cerdos)[13,14]. En España se han descrito infecciones por SARM-CO principalmente en niños y en pacientes de origen sudamericano[15-17].

Los clones de SARM-CO difieren notablemente de los SARM hospitalarios o SARM-ACS, y presentan genotipos distintos de los de aislados hospitalarios de la misma comunidad. Actualmente, la mayor incidencia de SARM-CO se ha observado en Estados Unidos, donde los clones más frecuentes son los denominados USA400 y USA300, pertenecientes a las secuencias tipo ST1 y ST8 de MLST *(multilocus sequence typing)*, respectivamente[4,8]. Estos 2 clones son los responsables de la mayoría de las infecciones causadas por SARM-CO, principalmente el USA300 (cepa tipo denominada USA300-0114) que actualmente ha desplazado al USA400, y que posteriormente se ha diseminado por los hospitales de Estados Unidos y también por Europa y Australia[8,18,19]. En otros continentes los ST más frecuentes de SARM-CO son el ST30 (Pacífico sur), el ST59 (Taiwán) y el ST80 (Europa). En Europa también se han descrito el ST8 y el ST30[5,20]. En España el clon más frecuente de SARM-CO pertenece al ST8 y está relacionado con el clon USA300; con menor frecuencia también se han detectado otros clones pertenecientes al ST80 y al ST5 (pediátrico)[16,17]. El ST398, que históricamente no se ha asociado a infección en humanos (clon de animales), es actualmente el SARM-CO más frecuente en granjeros en contacto con cerdos, e incluso en la población general en algunas regiones de Europa[13].

Características genéticas

Las características genéticas y fenotípicas de SARM-CO difieren de las de SARM-ACS. Todos los aislados de SARM presentan una isla genética móvil denominada el *cassette* cromosómico estafilocócico (SCC*mec*), en el que se localiza el gen *mecA*, determinante genético necesario para la expresión de la resistencia a la oxacilina y, en consecuencia, a todos los betalactámicos. Se han descrito distintos tipos de SCC*mec* con diferentes tamaños moleculares[11]. Los SCC*mec* de tipo IV (21-24 kb) y V (28 kb) son más pequeños que los de los tipos I, II y III (34, 53 y 67 kb, respectivamente) y, teóricamente, más fácilmente transferibles. Los SCC*mec* de los tipos I, II y III, además del gen *mecA*, contienen otros genes que codifican la resistencia a diferentes antimicrobianos no betalactámicos y se asocian a las cepas hospitalarias, de ahí el fenotipo de multirresistencia característico de las cepas nosocomiales. Por el contrario, los tipos IV y V generalmente sólo presentan el gen *mecA* y se asocian a las cepas de SARM-CO que son sensibles a múltiples antibióticos[11]. Recientemente, se ha descrito en Taiwán un nuevo tipo de SCC*mec* relacionado con SARM-CO, el SCC*mec* V_T[21]. Una hipótesis que podría explicar el origen de SARM-CO es que el gen *mecA* o el SCC*mec* se transfirieran horizontalmente a una o a más cepas de *S. aureus* sensibles a oxacilina que ocupaban nichos tradicionalmente comunitarios. Esta posibilidad explica las diferentes características genotípicas y fenotípicas del SARM-CO. El relativamente largo tiempo pasado entre la aparición de los SARM en los hospitales y su aparición en la comunidad puede ser debido, en parte, a la baja tasa de transferencia genética cromosómica horizontal[22].

En España, hasta el momento actual, todos los SARM-CO descritos presentan el SCC*mec* de tipo IV (con las variantes IVa y IVc); sin embargo, este tipo IV también es el más frecuente entre los aislados hospitalarios, por lo que la simple caracterización de este *cassette* no permite en nuestro medio diferenciar entre SARM-CO y SARM-ACS[15-17,23]. No obstante, las cepas de SARM-CO, en comparación con las cepas de SARM-ACS, presentan diferentes patrones de bandas en la electroforesis en campo pulsante y diferentes tipos de secuencia en el MLST. De este modo, en España, el genotipo de SARM-ACS más frecuente es el ST125-MRSA-IV[23], mientras que el de SARM-CO más frecuente es el ST8-MRSA-IV[16,17]. Aunque como se indicó anteriormente, el SCC*mec* de tipo IV no contiene otros determinantes de resistencia salvo el *mecA*, las cepas de SARM-CO pueden adquirir resistencia a múltiples antimicrobianos mediante la adquisición de plásmidos. Así, cada vez es más frecuente que presenten resistencia a la eritromicina y a la clindamicina por la adquisición de genes *erm* y *msrA*, o a las tetraciclinas por la adquisición de genes *tet*[4].

Toxinas y patogenia

Se ha indicado que el repertorio de genes que codifican las toxinas presentes en las cepas de SARM-CO puede contribuir a la diferencia en el espectro de enfermedad entre los SARM-CO y los SARM-ACS[6]. Hay 6 genes de exotoxinas que se han encontrado significativamente más en los SARM-CO, y 7 que son más frecuentes entre los SARM-ACS. Los más frecuentes entre los SARM-CO son: *lukS-PV/lukF-PV, sea, seb, sec, seh* y *sek*. No obstante, todavía no está claro si algunos de ellos confieren especiales características de virulencia a los SARM-CO[11]. Los 2 genes *lukS-PV/lukF-PV*, que codifican la producción de la leucocidina de Panton-Valentine (LPV), son particularmente frecuentes en SARM-CO, y tienen un papel importante en su virulencia. La LPV es una citotoxina que destruye la integridad de los leucocitos polimorfonucleares y produce necrosis tisular. Se cree que la toxina causa neutropenia y daño tisular, debido a la liberación de productos tóxicos por parte de los neutrófilos. Los genes de la toxina LPV se transfieren de una cepa a otra mediante bacteriófagos[11] y se insertan en un lugar específico del cromosoma distinto del lugar de inserción del SCC*mec*. La presencia de LPV en SARM-CO no parece ser necesaria para la colonización ni, por tanto, para la diseminación. Sin embargo, hay una gran correlación entre la presencia de los genes LPV y los aislados de SARM-CO productores de enfermedad. Los aislados de pacientes con fascitis y miositis necrosante y con neumonía necrosante, generalmente, presentan la toxina LPV, y el 77-100% de los aislados de SARM-CO productores de enfermedad contienen los genes de la toxina LPV[5,6,11]. Actualmente se considera que la LPV es un factor de virulencia implicado en infecciones de piel y partes blandas, neumonía necrosante e infecciones de huesos y articulaciones por SARM-CO, si bien la relación entre LPV y virulencia es independiente de la resistencia a la meticilina[11,24]. Los genes de la LPV se han encontrado en múltiples entornos genéticos de SARM-CO en todos los continentes, por lo que el incremento de la enfermedad por SARM-CO se debe a la diseminación de múltiples y diversos entornos genéticos de *S. aureus* más que a la diseminación de un único clon. Además, la unión del SCC*mec* y la LPV en una misma cepa parece conferir una ventaja selectiva para la patogenicidad[5]. Las cepas de SARM-CO productoras de LPV pertenecen a clones epidémicos que no están relacionados con los SARM-ACS, y probablemente por este motivo estos últimos no presentan la toxina LPV. Aunque la presencia de LPV es en general un marcador de SARM-CO, ésta no es una característica estrictamente necesaria, ya que algunos clones de SARM-CO no la producen[11]. Además de la toxina LPV, otro mecanismo de patogenicidad que presentan algunos clones de SARM-CO es la isla de patogenicidad denominada ACME *(arginine catabolic mobile element)*. Este elemento está presente en el clon USA300 y contribuye a su patogenicidad, aumentando el crecimiento y la supervivencia de este clon[25].

Características clínicas

El espectro clínico de SARM-CO es similar al de *S. aureus* sensible a la meticilina (SASM), e incluye la colonización asintomática, las infecciones de piel y partes blandas, y las infecciones invasivas[26]. No hay datos publicados acerca de la prevalencia de la colonización por SARM en la población general española, pero sí de Estados Unidos, donde se ha pasado del 0,8% en 2001-2002 al 1,5% en 2003-2004[27]. La colonización por SARM-CO, como ocurre también con el SARM-ACS, constituye un factor de riesgo para el desarrollo posterior de una infección, principalmente de piel y partes blandas[11]. La mayoría de las infecciones producidas por SARM-CO son leves, y se limitan a piel y partes blandas, principalmente forúnculos, carbunco (ántrax) y abscesos, que constituyen la presentación clínica más común. Las lesiones necróticas cutáneas se confunden con cierta frecuencia con picaduras de araña, debido a que presentan centros necrosados. En ocasiones, estas lesiones progresan a celulitis e incluso a infecciones invasivas y fatales como la fascitis necrosante[9,26]. La neumonía necrosante está asociada a cepas de SARM-CO productoras de LPV. Se produce más frecuentemente en niños y jóvenes, y se caracteriza por fiebre alta, hemoptisis, hipotensión, leucopenia e infiltrados alveolares difusos que evolucionan a abscesos. La mortalidad es extremadamente alta y, en muchas ocasiones, la neumonía necrosante se produce como complicación de una infección viral previa, principalmente gripe[11]. Otras presentaciones clínicas descritas son bacteriemia, shock séptico acompañado del síndrome de Waterhouse-Friderichsen, tromboflebitis, endocarditis, artritis, osteomielitis, endoftalmitis y mediastinitis[9].

Diagnóstico microbiológico

Las cepas de SARM-CO presentan, típicamente, una serie de características microbiológicas que las diferencian de los aislados de SARM-ACS: ser resistentes sólo a los antibióticos betalactámicos (generalmente con una expresión heterogénea de la resistencia a la oxacilina, lo que en ocasiones dificulta su detección en el laboratorio); ser portadoras del SCC*mec* de tipo IV o V, contener los

genes *lukF-PV* y *lukS-PV* que codifican la toxina LPV, y poseer patrones genotípicos diferenciados en la electroforesis en campo pulsante y en el MLST[6]. No obstante, es preciso matizar algunas de estas peculiaridades. En la práctica habitual del laboratorio, una cepa de *S. aureus* será con gran probabilidad un SARM-CO cuando en el antibiograma se observe una resistencia exclusiva a los betalactámicos, generalmente con heterorresistencia a la oxacilina (crecimiento de colonias de *S. aureus* en el interior del halo de la oxacilina), y con sensibilidad a múltiples clases de antimicrobianos, especialmente a ciprofloxacino, ya que la casi totalidad de las cepas de SARM-ACS en España son resistentes a este fármaco. Ahora bien, el clon de SARM-CO predominante en Estados Unidos, USA300 (ST8-MRSA-IV), presenta una elevada tasa de resistencia a los macrólidos[7], pero no a la clindamicina. Sin embargo, muchas cepas sensibles *in vitro* a este último compuesto contienen genes que codifican la resistencia inducible a los macrólidos, lincosamidas y estreptogramina B y que confieren el potencial de desarrollar resistencia a la clindamicina durante el tratamiento. Por lo tanto, ante la sensibilidad a la clindamicina, siempre se debe realizar en el laboratorio la técnica de difusión con doble disco *(D-zone test)* para detectar la posible presencia de resistencia inducible. El clon europeo (ST80-MRSA-IV) es resistente a la tetraciclina, la kanamicina y al ácido fusídico[28]. Además, mediante la adquisición de plásmidos también se produce con cierta frecuencia la resistencia a otros antimicrobianos[7]. En España es frecuente que los aislados de SARM-CO sean resistentes a la tetraciclina y a la doxiciclina, resistencia mediada por el gen *tet*(M)[17]. Respecto a la presencia del SCC*mec* de tipo IV en los SARM-CO, ya se indicó anteriormente que esta característica apenas tiene utilidad para diferenciarlos de los SARM-ACS, debido a que la mayoría de las cepas hospitalarias en nuestro país presenta este tipo de *cassette*[23].

En cuanto a la toxina LPV, hay una asociación entre las cepas de SARM-CO aisladas de forúnculos, abscesos cutáneos y neumonías necrosantes y la producción de dicha toxina[24], pero no todos los aislados de SARM-CO la producen. En un estudio reciente, los genes de la LPV se detectaron en el 98% de aislados de pacientes con infecciones de piel y partes blandas en Estados Unidos[7]. Ya se ha indicado anteriormente que las cepas de SARM-CO, en comparación con las cepas de SARM-ACS, presentan un patrón diferente de bandas en la electroforesis en campo pulsante y una secuencia tipo diferente en el MLST. No obstante, en los últimos años, muchos de estos aislados de SARM-CO se han introducido en los hospitales y se detectan también en pacientes ingresados, con lo que la distinción entre SARM-ACS y SARM-CO, en un futuro, será cada vez más difícil[8].

Tratamiento

Tradicionalmente, los glucopéptidos (vancomicina y teicoplanina) han sido el tratamiento de elección en las infecciones por SARM, debido a las escasas alternativas terapéuticas existentes por la multirresistencia de este microorganismo. Sin embargo, las cepas de SARM-CO suelen ser sensibles a otros muchos antimicrobianos, con lo que hay más opciones terapéuticas. En los pacientes ingresados con una infección grave por SARM-CO, la vancomicina continúa siendo el tratamiento de primera elección[29], con la excepción de las neumonías, en las que no se debería administrar vancomicina como tratamiento antibiótico único por su limitada penetración en el tejido pulmonar. Se desconoce si en estos casos la adición de clindamicina o linezolid (que pueden disminuir la producción de la LPV), o de inmunoglobulina intravenosa (que contiene anticuerpos frente a la LPV), tiene algún efecto en la evolución[30]. No obstante, la mayoría de cepas de SARM-CO se detectan en pacientes ambulatorios con una infección leve de piel y partes blandas, que no requieren ingreso hospitalario. El tratamiento único con incisión y drenaje puede ser adecuado en niños y pacientes jóvenes con abscesos cutáneos no complicados y sin signos de infección sistémica. En situaciones en las que el paciente no responde a un drenaje adecuado, el tratamiento consiste en la administración de antibióticos orales, con o sin incisión más drenaje. La administración de un antibiótico, junto con el drenaje, está recomendada en casos de gravedad de los síntomas locales, presencia de signos o síntomas de infección sistémica, comorbilidad asociada, inmunosupresión y en los extremos de edad[9,26]. Las cefalosporinas orales y las penicilinas antiestafilocócicas, que han sido la base del tratamiento de las infecciones de piel y partes blandas en la comunidad, son inactivas frente a SARM-CO; por lo tanto, el principal reto en el tratamiento empírico es sospechar la presencia de este microorganismo y utilizar diferentes alternativas terapéuticas. Entre éstas, la clindamicina constituye probablemente el tratamiento empírico de elección y se ha mostrado eficaz para el tratamiento de las infecciones causadas por aislados de SARM-CO[31]. Penetra adecuadamente en los tejidos, incluyendo pulmón, líquido pleural, tejido subcutáneo y hueso, y tiene la ventaja adicional de que, al actuar en el ribosoma bacteriano, inhibe la producción de toxinas, como la LPV. Se han descrito fracasos en pacientes que recibieron clindamicina para el tratamiento de infecciones causadas por aislados con resistencia inducible[31]. En consecuencia, en las cepas resistentes a la eritromicina y sensibles a la clindamicina, se debe realizar el *D-zone test*. La combinación trimetoprima-sulfametoxazol (cotrimoxazol) tiene una excelente actividad bactericida frente cepas de SARM-CO[11] y constituye una buena alternativa, pero no se han realizado ensayos clínicos amplios. Las tetraciclinas (doxiciclina o minociclina) son una opción razonable para el tratamiento de las infecciones de piel y partes blandas, pero no en infecciones sistémicas, ya que hay una tasa de fracasos inaceptable[11]. Además, hay que tener en cuenta que, en el caso de resistencia mediada por *tet*(M), hay resistencia a la doxiciclina, y que este hecho es relativamente frecuente en los SARM-CO aislados en España[17]. La utilización de rifampicina en monoterapia genera resistencia[11], por lo que, en caso de utilizarse, se debe asociar con otro antimicrobiano. En cuanto a las fluoroquinolonas, en los últimos años se ha producido un incremento en la resistencia al ciprofloxacino en cepas de SARM-CO en Estados Unidos[7], y actualmente no deben constituir una elección prioritaria. Habitualmente se desarrolla resistencia a las quinolonas durante el tratamiento, por mutaciones en la ADN girasa, si bien las nuevas quinolonas, como el moxi-

floxacino, tienen una menor capacidad de inducir resistencias. Por otra parte, varios estudios indican que el tratamiento previo con ciprofloxacino o levofloxacino puede favorecer la colonización por SARM[32]. El linezolid tiene una eficacia superior a la vancomicina en la neumonía nosocomial por SARM y su uso debería limitarse a infecciones graves por SARM-CO, especialmente neumonías, debido a su elevado coste y a su toxicidad con el uso prolongado[29]. Al igual que la clindamicina, tiene la ventaja de disminuir la producción de la LPV. Entre los nuevos antimicrobianos, la daptomicina se ha mostrado eficaz para el tratamiento de infecciones por SARM-CO[33], aunque no se puede utilizar en el tratamiento de la neumonía, y la tigeciclina presenta actividad frente a aislados de SARM-CO pero no hay experiencia clínica[34].

Colonización y prevención

El cultivo nasal para el estudio de la colonización por SARM se realiza ampliamente en los hospitales para el control de la infección por este microorganismo, pero en el caso de SARM-CO, la infección no está necesariamente precedida de la colonización nasal, por lo que es más difícil identificar y controlar poblaciones que están en riesgo de desarrollar una infección por este microorganismo. La colonización también puede ser gastrointestinal, lo que representaría una forma diferente de diseminación, y además los animales de compañía pueden ser también reservorios adicionales[12,35]. En el momento actual, el conocimiento de la epidemiología de SARM-CO es incompleto, y el reto más importante es controlar la infección. En nuestro medio, dado que la incidencia de infecciones por SARM-CO es actualmente muy baja, lo más importante es su reconocimiento mediante la realización de cultivo y antibiograma de todos los abscesos e infecciones de piel adquiridos en la comunidad y no asumir que se pueda tratar de un aislado de *S. aureus* sensible a la meticilina. Por otra parte, como el contacto de piel con piel es el principal mecanismo de transmisión de una cepa colonizadora o productora de infección entre un huésped y otro, las medidas de prevención deben dirigirse a recalcar la importancia de las medidas de higiene general para reducir la transmisión. Éstas incluyen minimizar el riesgo de traumatismos cutáneos (como el uso de protectores tipo rodillera y calcetines durante algunas actividades deportivas), mantener las heridas limpias y tapadas con vendajes secos, lavado de manos e higiene corporal frecuentes, evitar compartir toallas o ropas que se hayan puesto en contacto directo con la piel y la eliminación adecuada de los objetos contaminados[11,26]. En las personas no ingresadas, el tratamiento descolonizador de los pacientes o de sus contactos sólo está recomendado en dos situaciones: en pacientes con infecciones recurrentes por SARM-CO y ante un brote por SARM-CO en una comunidad bien definida (p. ej., en una familia)[29]. El tratamiento descolonizador con mupirocina nasal y clorhexidina corporal durante 5 días se ha mostrado eficaz para el control de un brote de SARM-CO en Dinamarca en 22 de 23 pacientes[28] pero, en general, no hay suficientes datos que apoyen la utilización de agentes antimicrobianos o antisépticos para eliminar la colonización. Actualmente no hay ninguna vacuna frente a *S. aureus* disponible, aunque hay varias en investigación con resultados prometedores[36].

Conclusiones

En la última década se ha producido un aumento de las infecciones por SARM en la comunidad cuyo origen no está relacionado con el hospital o con los cuidados sanitarios. Estas infecciones producidas por SARM-CO en pacientes que no presentan los factores de riesgo establecidos para la infección por SARM constituyen actualmente una epidemia en países como Estados Unidos. Aunque estos microorganismos generalmente producen infecciones leves de piel y partes blandas, también son agentes causales de neumonía necrosante, sepsis y otras enfermedades invasivas. Las cepas de SARM-CO tienen elementos genéticos y toxinas distintos de los de SARM-ACS, y estas diferencias se consideran las causas de las variaciones en la epidemiología y en la enfermedad. La detección precoz de la enfermedad por SARM-CO es crucial, pero también difícil. En España, donde la presencia de SARM-CO es todavía poco frecuente, lo más importante es su reconocimiento mediante la realización de cultivo y antibiograma de todos los abscesos e infecciones de piel adquiridos en la comunidad, con objeto de conocer la etiología y guiar el tratamiento. Actualmente, las medidas de higiene y el estricto cumplimiento de las precauciones de contacto son las medidas más eficaces para evitar la diseminación de SARM-CO.

Declaración de conflicto de intereses

Los autores han declarado no tener ningún conflicto de intereses.

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Natural history of meticillin-resistant *Staphylococcus aureus* colonisation among residents in community long term care facilities in Spain

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SUMMARY

The spread of meticillin-resistant *Staphylococcus aureus* (MRSA) is a major problem for both acute care hospitals and among residents in long term care facilities (LTCFs). We performed a cohort study to assess the natural history of MRSA colonisation in LTCF residents. Two cohorts of residents (231 MRSA carriers and 196 non-carriers) were followed up for an 18 month period, with cultures of nasal and decubitus ulcers performed every six months. In the MRSA carrier cohort, 110 (47.8%) residents had persistent MRSA colonisation for six months or longer, 44 (19.0%) had transient colonisation and nine (3.9%) were intermittently colonised. No risk factors for persistent MRSA colonisation could be determined. The annual incidence of MRSA acquisition was around 20% [95% confidence interval (CI): 14.3–25.5]. Antibiotic treatment was independently associated with MRSA acquisition (odds ratio: 2.27; 95% CI: 1.05–4.88; $P = 0.03$). Just two clones were distinguishable by pulsed-field gel electrophoresis and multilocus sequence typing: CC5-MRSA IV, which is widely disseminated in Spanish hospitals, and ST22-MRSA IV. This study adds to the knowledge of the epidemiology of MRSA in community LTCFs, which are important components of long term care in Spain.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) is endemic in most acute care hospitals and long term care facilities (LTCFs) in Spain and is a major problem for the healthcare system.[1–4] Recently we found a 17% prevalence of MRSA colonisation in Spanish community LTCFs.[3] In this cross-sectional study of *S. aureus* colonisation, we found comorbidity and size of LTCFs to be associated with meticillin-susceptible *S. aureus* (MSSA) colonisation; and comorbidity, age, hospital admissions, medical devices, decubitus ulcers and antibiotic use associated with MRSA colonisation.[3] Other risk factors for nasal *S. aureus* colonisation among nursing home residents are indwelling devices, functional dependence, low nursing staff ratio, location of LTCF in a deprived area, and prolonged hospitalisation in the previous two years.[5–7] There are limited data on the natural history of MRSA colonisation in LTCFs.[6,8,9]

We conducted a multicentre cohort study among residents of community LTCFs for the elderly to further define the natural history of MRSA colonisation.

Methods

Study population and characteristics of community LTCFs

The characteristics of this population have been described.[3] Nine community LTCFs for the elderly, located in two

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communities in Spain (Catalonia and Balearic Islands) with 1586 beds (median: 120; range: 72–552) were included. Five were located in the catchment area of a 900 bed acute-care hospital (Hospital Universitari de Bellvitge, HUB), three in that of a 490 bed hospital (Corporació Sanitària Parc Taulí) and one in that of an 800 bed hospital (Hospital Universitari Son Dureta, HUSD). These facilities provide care for elderly long term residents, who may be disabled or infirm. Each LTCF has a dementia ward and its own medical staff. Residents are accommodated in rooms with one, two or three beds. Surveillance for MRSA and decolonisation procedures are not routine. In addition to standard precautions for all patient care, contact precautions are applied for residents colonised or infected with MRSA. Known MRSA carriers are not denied admission.[10]

Study design

This was a multicentre prospective cohort study conducted from November 2005 to May 2007. The study population consisted of all the residents in the LTCFs at baseline ($N = 1377$), and was identical to that of our previously published cross-sectional study.[3] A total of 231 residents were found to be colonised with MRSA at baseline (MRSA carrier cohort). A representative sample of non-MRSA carriers was selected from the 1146 residents without MRSA colonisation at baseline as follows: for each MRSA carrier identified at baseline, one non-MRSA carrier was randomly selected from the same ward and screened six months later. Those with two consecutive negative cultures were included in the non-MRSA carrier cohort ($N = 196$). Subjects were visited by the investigators every six months for an 18 month period (Figure 1). During this period no changes were made to infection control practices in the LTCFs and data from the study were not available to clinical staff.

Data collected and definitions

Complete clinical data were obtained for all residents at baseline and medical charts were reviewed thereafter at six-monthly intervals. Decubitus ulcers, hospital admissions, antibiotic consumption and clinical infections were recorded.

In the MRSA carrier cohort, persistent colonisation was defined as at least two MRSA-positive cultures separated by fewer than two negative cultures. Intermittent colonisation was defined as a second positive culture separated from the first by at least two negative cultures. Transient colonisation was defined as two or more negative cultures after a single positive culture for MRSA.

The prevalence of MRSA colonisation in each LTCF at baseline (17%; range: 6.7–35.8%) was used as surrogate for colonisation pressure.[3] High colonisation pressure was defined as prevalence of MRSA colonisation of >20%.[11,12]

Microbiological methods

Every six months we obtained nasal and where applicable decubitus ulcer swabs for culture. Areas were swabbed with sterile cotton-tipped applicator sticks and placed into Stuart transport medium. Swabs were plated on to coagulase mannitol agar and selective MRSA agar (MRSA Select, Bio-Rad Laboratories, Madrid, Spain), then inoculated into staphylococcal enrichment broth composed of brain–heart infusion plus 7% NaCl. After 24 h of incubation at 35 °C, broths were subcultured on to coagulase

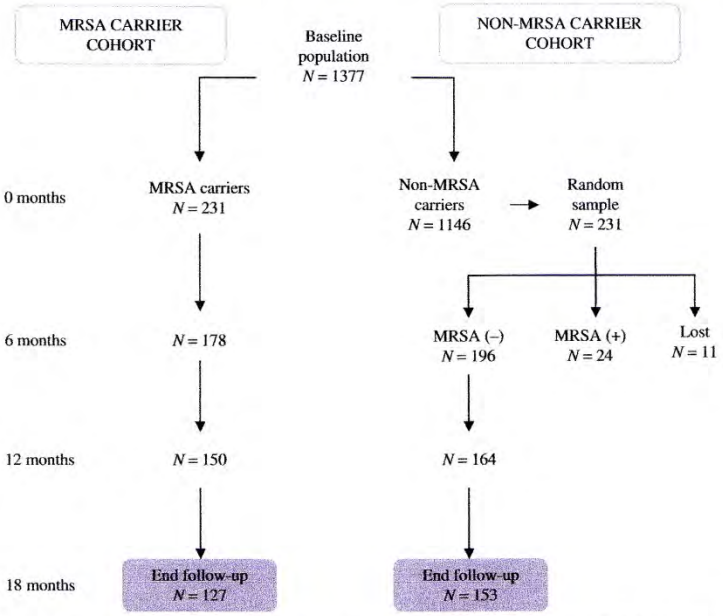

Figure 1. Residents considered and finally included at baseline and follow-up. MRSA, meticillin-resistant *Staphylococcus aureus*.

mannitol agar and selective MRSA agar. Plates were incubated for 48 h and inspected daily. Putative *S. aureus* colonies were identified by a latex agglutination test (Pastorex® Staph-plus, Bio-Rad) and DNase production (DNase Test Agar, Biomedics, Madrid, Spain). Testing for antimicrobial susceptibility was performed by the disc-diffusion method. Meticillin resistance was determined by cefoxitin disc diffusion following Clinical and Laboratory Standards Institute recommendations.[13]

Molecular epidemiology methods

Macrorestriction of chromosomal DNA and pulsed-field gel electrophoresis (PFGE) analysis were performed on MRSA isolates identified in the microbiology laboratories of HUB and HUSD. Genomic DNA was digested with *Sma*I and restriction fragments were separated using a CHEF DR III apparatus (Bio-Rad) following published methodology, and interpreted according to Van Belkum.[14,15] In a collection of representative isolates selected by their diverse PFGE patterns the following molecular studies were performed: staphylococcal cassette chromosome *mec* (SCC*mec*) polymorphism, genes encoding class S (*lukS-PV*) and class F (*lukF-PV*) proteins for Panton–Valentine leucocidin (PVL), and multilocus sequence typing (MLST).[16–18]

Statistical analysis

Categorical variables were analysed with χ^2-test or Fisher's test as appropriate and continuous variables by Student's *t*-test or non-parametric tests. Logistic regression models were used to analyse risk factors for persistence in the MRSA carrier cohort and risk factors for acquisition in the non-MRSA carrier cohort. Variables with $P < 0.15$ were included in the model. All statistical tests were two-tailed and $P < 0.05$ was deemed significant. SPSS package version 12.0 was used.

Approval for study was obtained from the Research Ethics Committee of HUB. No written informed consent was obtained since the study met criteria for a waiver of this requirement.

Results

Natural history of MRSA colonisation in the cohort colonised at baseline

A total of 231 residents colonised with MRSA at baseline were included. Of these, 104 were lost to follow-up; 70 died, 21 were discharged and 13 lost for other reasons. Table I compares residents who were lost to follow-up with those in this cohort followed to completion. Figure 2 shows the natural history of MRSA colonisation in the MRSA carrier cohort. We identified 110 (47.8%) persistent carriers, 44 (19.0%) transient carriers and nine (3.9%) intermittent carriers. The remaining 68 (29.4%) could not be classified under the definitions used.

Table I
Baseline characteristics of patients who did and did not complete the follow-up period in the MRSA carrier cohort

Characteristic	Completed follow-up $N = 127$	Lost to follow-up $N = 104$	*P*
Female sex	88 (69.3%)	71 (68.3%)	0.87
Age (years)[a]	81.6 (12.6)	83.3 (10.1)	0.52
Charlson index ≥2	50 (39.4%)	53 (50.9%)	0.08
Barthel index <30	49 (38.6%)	50 (48.1%)	0.15
Medical devices	10 (7.9%)	21 (20.2%)	0.006
Decubitus ulcers	30 (23.6%)	28 (26.9%)	0.56
LTCFs with <150 beds	56 (44.1%)	37 (35.6%)	0.19
LTCFs with prevalence of MRSA >20%	82 (64.6%)	55 (52.9%)	0.07
Two-site colonisation	17 (13.4%)	17 (16.3%)	0.53

MRSA, meticillin-resistant *Staphylococcus aureus*; LTCF, long term care facility.
[a] Mean (SD).

In order to identify risk factors for persistence, persistent carriers were compared with transient and intermittent carriers (Table II). Only residents with decubitus ulcers were significantly more likely to be persistent MRSA carriers, but this was not statistically significant in multivariate analysis.

Incidence of MRSA colonisation in the cohort not colonised at baseline

Among 196 residents with negative cultures for MRSA at baseline and at 6 months, 43 were colonised with MSSA and 153 were not colonised with *S. aureus*. Table III compares characteristics of residents in the non-MRSA carrier cohort who were lost to follow-up with those who were followed to completion of the study. Thirty-nine residents acquired MRSA while in the LTCF. The incidence of MRSA colonisation during LTCF stay was 19.9% [95% confidence interval (CI): 14.3–25.5] per year. Antibiotic use was independently associated with acquisition of MRSA (odds ratio: 2.12; 95% CI: 1.05–4.45; $P = 0.05$) (Table IV).

Molecular epidemiology

Among 491 residents of five LTCFs related to HUB, MRSA was isolated from 144 (276 MRSA isolates), 108 of whom were identified at baseline. All 276 MRSA isolates were studied by PFGE and 88% belonged to a single clone. Representative strains of this dominant clone belonged to ST146, single locus variant by MLST typing of ST5, founder of clonal complex 5. All belonged to SCC*mec* type IV and were PVL negative. Among 466 residents of one LTCF related to HUSD, 39 MRSA isolates were identified at baseline. Two different strains, clone A (three subtypes) and clone C (five subtypes) were recognised by PFGE. Clone A belongs to ST125 MRSA-IV and clone B to ST22-MRSA IV. All strains were PVL negative and carried SCC*mec*IV. Subsequent MRSA isolates had the same PFGE profiles as the initial isolates.

Discussion

We have previously reported a prevalence of MRSA colonisation of ~17% among residents of community LTCFs in Spain.[3] This is similar to reports from other European countries, but is lower than the prevalence reported in Veterans Affairs facilities in the USA.[7,19–22] There is little information on the dynamics of MRSA colonisation in residents of community LTCFs.

Most of the carriers we identified had persistent MRSA colonisation. This agrees with studies gathered in Veterans Affairs facilities, albeit with different epidemiological features.[6] We did not identify any factor that distinguished persistent MRSA carriers from transient and intermittent carriers. Stone *et al.* found that only bacterial burden could distinguish the groups.[23] Defining the duration of colonisation is problematic, because low bacterial burdens lead to false-negative results and the limited number of samples collected from residents could contribute towards the misclassification of colonisation status.

There was a high incidence of MRSA colonisation in this study, ~20% per annum. Only antibiotic use was independently associated with MRSA acquisition in LTCFs. We used the prevalence of MRSA colonisation in each LTCF at baseline as a surrogate for colonisation pressure. This is regarded as an important determinant of the probability of acquisition when organisms spread mainly via cross-colonisation.[11,12] In our population, the probability of MRSA

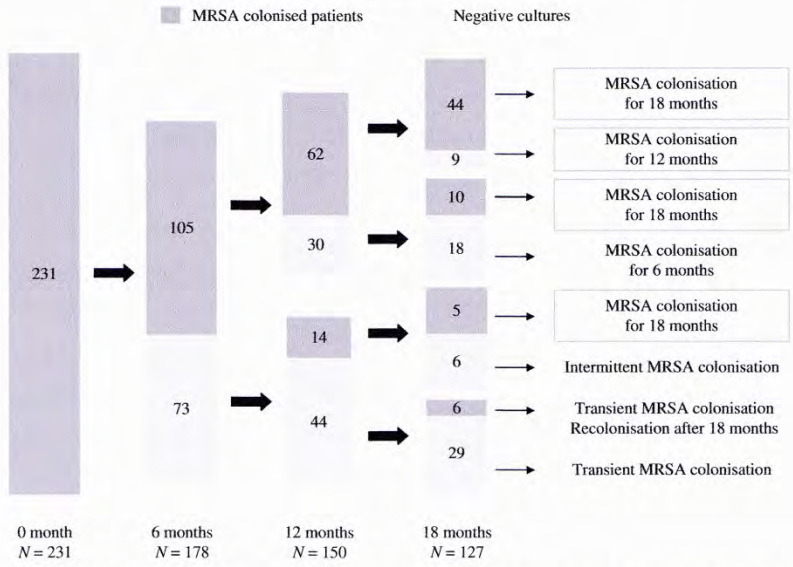

Figure 2. Natural history of meticillin-resistant *Staphylococcus aureus* (MRSA) colonisation in the MRSA carrier cohort. Results of MRSA detection performed at each point of the study. Subjects lost to follow-up are not included.

acquisition was no greater in LTCFs with a high baseline MRSA prevalence, which we defined as >20%. Perhaps the baseline prevalence of MRSA colonisation in an LTCF did not represent adequately the colonisation pressure throughout the study period.

The spread of MRSA in community LTCFs increases the reservoir of this organism in the healthcare setting, and residents transferred from LTCFs account for a large part of the burden of hospitalised MRSA carriers. Programmes for detection of carriers at hospital admission should take account of this.[24–26]

Regarding the molecular epidemiology results, ST146 and ST125 are single locus variants of ST5, and therefore both belong to CC5. Remarkably, only two distinct clones could be distinguished by PFGE and MLST: CC5-MRSA IV and ST22-MRSA IV. The former has been reported to be widely disseminated in Spanish hospitals.[27,28] The dominance of clone ST146-MRSA-IV made it impossible to differentiate between persistent colonisation and recurrent acquisition.

Our study has a number of limitations. Apart from nasal swabs, we cultured swabs only from decubitus ulcers, the most frequent skin lesion. High levels of MRSA colonisation in decubitus ulcers have been reported among LTCF residents.[9,21,29] However, recent results suggest that most community LTCF residents with MRSA are colonised at multiple sites. Some colonised residents are not detected unless multiple sites are sampled and one-third are missed if only a nasal swab is collected.[8] We did not collect data on potential confounders such as nursing staff:bed ratio, invasive devices or functional status of residents. Functional status is likely to have been poorer in those lost to follow-up, because deaths

Table II
Risk factors for persistent MRSA colonisation among residents in community long term care facilities

	Transient or intermittent colonisation $N=53$	Persistent MRSA colonisation $N=110$	Bivariate analysis P	Logistic regression OR (95% CI)
Aged >85 years	22 (41.5%)	45 (40.9%)	0.94	0.99 (0.96–1.02)
Female sex	43 (81.1%)	73 (66.4%)	0.06	2.02 (0.91–4.50)
Charlson index ≥2	16 (30.8%)	46 (42.6%)	0.15	0.55 (0.28–1.08)
Barthel index <30	23 (34.8%)	43 (39.8%)	0.59	
Decubitus ulcers	7 (13.7%)	30 (27.5%)	0.05	0.51 (0.22–1.15)
Two-site colonisation	3 (9.7%)	17 (19.1%)	0.23	
Hospital admissions	6 (11.5%)	23 (21.1%)	0.14	0.61 (0.55–1.44)
Antibiotic use	28 (53.8%)	67 (61.5%)	0.36	
LTCFs with MRSA colonisation prevalence >20%	30 (57.7%)	73 (67.0%)	0.25	

MRSA, meticillin-resistant *Staphylococcus aureus*; OR, odds ratio; CI, confidence interval; LTCF, long term care facility.

Table III
Baseline characteristics of residents who did and did not complete follow-up in the non-MRSA carrier cohort

Characteristic	Completed follow-up $N=153$	Lost to follow-up $N=43$	P
Female sex	103 (67.3%)	31 (72.1%)	0.55
Age (years)[a]	80.4 (11.1)	83.4 (11.6)	0.89
Charlson index ≥2	49 (32.0%)	17 (39.5%)	0.36
Barthel index <30	52 (34.2%)	25 (58.1%)	0.005
Decubitus ulcers	10 (6.5%)	7 (16.3%)	0.05
LTCFs with <150 beds	53 (34.6%)	23 (46.5%)	0.16
LTCFs with MRSA prevalence >20%	90 (58.8%)	33 (76.7%)	0.03

MRSA, meticillin-resistant *Staphylococcus aureus*; LTCF, long term care facility.
[a] Mean (SD).

Table IV
Risk factors associated with MRSA acquisition in community long term care facilities

	New MRSA carriers $N = 39$	Non-MRSA carriers $N = 157$	Bivariate analysis P	Logistic regression OR (95% CI)
Age (years)[a]	78.6 (12.3)	81.6 (10.9)	0.88	0.98 (0.95–1.01)
Female sex	24 (61.5%)	110 (70.1%)	0.31	0.74 (0.34–1.64)
Charlson index ≥2	14 (35.9%)	55 (33.1%)	0.73	
Barthel index <30	18 (46.2%)	59 (37.8%)	0.34	
Decubitus ulcers	6 (15.4%)	11 (7.0%)	0.09	2.17 (0.73–6.52)
Hospital admissions	6 (15.4%)	21 (13.4%)	0.75	
Antibiotic use	25 (64.1%)	69 (43.9%)	0.02	2.12 (1.05–4.45)
LTCFs with MRSA prevalence >20%	23 (59.0%)	100 (63.7%)	0.59	

MRSA, meticillin-resistant *Staphylococcus aureus*; OR, odds ratio; CI, confidence interval; LTCF, long term care facility.
[a] Mean (SD).

accounted for most of them, and poor functional status is associated with earlier death in this population.[30]

Few longitudinal studies have assessed the incidence of MRSA colonisation among residents of LTCFs.[6] This multicentre prospective study adds to existing knowledge of natural history of MRSA in community LTCFs and may inform more effective control measures.

Conflict of interest statement
None declared.

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Original

Diseminación nosocomial de *Staphylococcus hominis* resistente al linezolid en dos hospitales de Mallorca

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RESUMEN

Introducción: A partir del 2008 se detectaron varios aislados de *Staphylococcus hominis (S. hominis)* multirresistentes, incluyendo resistencia al linezolid y a la teicoplanina, en pacientes ingresados en dos hospitales de Mallorca. Por ello, se inició un estudio para determinar la epidemiología molecular y el mecanismo de resistencia al linezolid.

Métodos: El estudio de epidemiología molecular se realizó mediante electroforesis en campo pulsado (ECP), tras digestión con ApaI. Se efectuó amplificación de un fragmento de los genes ARNr 23S (con secuenciación posterior) y *cfr*.

Resultados: Desde marzo de 2008 hasta febrero de 2009 se detectaron 15 aislados de *S. hominis* resistentes al linezolid y a la teicoplanina, procedentes de 14 pacientes. Todos ellos excepto uno habían ingresado en las Unidades de Cuidados Intensivos de alguno de los dos hospitales. La mayoría de los aislados (9) se obtuvieron en hemocultivos. Gran parte de los pacientes infectados (12 de los 15 episodios infecciosos, el 80,0%) recibieron pautas de linezolid antes de la detección del aislado resistente. La ECP reveló la presencia de un único clon entre los aislados de *S. hominis* resistentes al linezolid. Se detectó la mutación G2576T en todas las cepas resistentes, mientras que la PCR del gen *cfr* fue negativa en las mismas. Todos los aislados fueron también resistentes a la penicilina, oxacilina, trimetoprim-sulfametoxazol, ciprofloxacino, levofloxacino y tobramicina; y sensibles a la eritromicina, tetraciclina, gentamicina y daptomicina. La CMI a la vancomicina fue de 4 μg/ml en todos ellos.

Conclusiones: La detección de cepas de estafilococos resistentes al linezolid resalta la necesidad de racionalizar el uso del linezolid y mantener un control activo de dicha resistencia con objeto de preservar la utilidad clínica de este antimicrobiano.

Nosocomial spread of linezolid-resistant *Staphylococcus hominis* in two hospitals in Majorca

Keywords:
Staphylococcus
Linezolid resistance
23S rRNA

ABSTRACT

Objective: Since March 2008, several linezolid and teicoplanin-resistant *Staphylococcus hominis (S. hominis)* isolates have been recovered from patients admitted to the two major hospitals on the island of Majorca, Spain. For this reason, a study was conducted to determine the molecular epidemiology of these isolates and the mechanism of linezolid resistance.

Methods: The molecular epidemiology study was performed by pulsed-field gel electrophoresis (PFGE) analysis, after digestion with ApaI. Linezolid resistance mechanisms were evaluated by PCR amplification of a fragment of the domain V of the 23S rRNA gene (followed by sequencing) and *cfr* gene.

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Results: From March 2008 to February 2009, 15 linezolid and teicoplanin-resistant *S. hominis* isolates were recovered from 14 patients. All of them, except one, were hospitalised in the intensive care units of either of the two institutions. Isolates were obtained mainly from blood cultures (9). The majority of infected patients (12 of 15 infectious episodes, 80.0%) had received courses of linezolid prior to detection of the resistant isolate. PFGE analysis revealed the presence of a unique clone among linezolid resistant *S. hominis* isolates. The G2576T mutation was detected in all the linezolid resistant strains. None of the resistant isolates showed a positive PCR for the *cfr* gene. All of the isolates were also resistant to penicillin, oxacillin, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, and tobramicin; whereas all of them were susceptible to erythromycin, tetracycline, gentamicin, and daptomycin. The MIC of vancomycin was 4 μg/ml for all the strains.
Conclusions: The detection of linezolid resistant Staphylococci highlights the need to rationalise the use of linezolid, and maintain an active surveillance of its resistance to preserve the clinical usefulness of this antimicrobial.

Introducción

Los estafilococos coagulasa negativa (SCN) se encuentran frecuentemente como residentes ubicuos en la piel y membranas mucosas de personas sanas. Sin embargo, constituyen también la principal causa de bacteriemia y de infecciones relacionadas con catéteres, especialmente en los pacientes ingresados en las Unidades de Cuidados Intensivos (UCI)[1,2]. En España, en dos estudios multicéntricos, entre los aislados clínicamente significativos de SCN, *Staphylococcus hominis (S. hominis)* ocupó el segundo lugar en frecuencia tras *Staphylococcus epidermidis*[3,4].

La mayoría de aislados de SCN son habitualmente resistentes a múltiples antibióticos, incluyendo las penicilinas resistentes a penicilinasa[5]. Por ello, se recomienda el tratamiento empírico con glucopéptidos (vancomicina o teicoplanina) para cubrir las infecciones producidas por estos microorganismos. No obstante, la aparición de cepas de SCN con sensibilidad disminuida a los glucopéptidos (incluyendo aislados resistentes a glucopéptidos), los parámetros farmacocinéticos subóptimos para este grupo antibiótico y la toxicidad limitan su utilidad[6]. Por todo ello se ha considerado la administración de otras familias de antimicrobianos, entre ellas las oxazolidinonas. El mecanismo de acción de las oxazolidinonas se basa en la unión a la subunidad 50S del ribosoma bacteriano (concretamente al dominio V del ARNr 23S), inhibiendo la síntesis proteica[7]. El linezolid es, hasta el momento, la única oxazolidinona aprobada para su uso.

La resistencia al linezolid en SCN es extremadamente rara, aunque incrementándose en los últimos años. En un informe que recoge la resistencia al linezolid en cepas de grampositivos detectadas en el año 2008 (Zyvox® *Annual Appraisal of Potency and Spectrum* [ZAAPS]), en el que participaron 24 países del mundo, entre 748 aislados de SCN, solamente 3 de ellos (0,4%) fueron resistentes al linezolid[5]. El mecanismo de resistencia al linezolid más frecuentemente detectado en estafilococos se produce por mutaciones en el dominio V del gen ARNr 23S, mayoritariamente la mutación G2576T (según la numeración para *Escherichia coli [E. coli]*)[5,7–9]. La resistencia al linezolid también puede producirse por mutaciones en las proteínas ribosomales L3 o L4, y por la presencia del gen *cfr*, que forma parte de un plásmido que puede ser horizontalmente transferido a otros estafilococos[10].

Desde marzo del 2008, se detectaron los primeros aislados de *S. hominis* resistentes al linezolid y a la teicoplanina, en dos hospitales terciarios de Mallorca, principalmente a partir de hemocultivos de pacientes hospitalizados en UCI. Este trabajo se llevó a cabo con el fin de investigar el mecanismo de resistencia y la epidemiología molecular de los aislados de *S. hominis* resistentes al linezolid detectados en los dos hospitales desde marzo de 2008 hasta febrero de 2009.

Material y métodos

Hospitales participantes

Este estudio fue llevado entre marzo de 2008 y febrero de 2009 en los dos principales hospitales públicos de la isla de Mallorca, España. El Hospital Son Llàtzer (HSLL) es un hospital terciario con 377 camas. La UCI del HSLL es una unidad médico-quirúrgica con 18 camas. El Hospital Universitario Son Dureta (HUSD) es el hospital terciario de referencia para las Islas Baleares con 740 camas. La UCI del HUSD tiene 30 camas divididas en 5 unidades según enfermedad Coronaria, Médica, Quirúrgico-Traumatológica, Neurocrítica y de Cirugía Cardíaca.

Identificación bacteriana

Los aislados se identificaron mediante el sistema Vitek® 2 (bioMérieux, Francia) en HSLL o API® ID 32 STAPH (bioMérieux, Francia) en HUSD. Para confirmar la identificación de *S. hominis*, se realizó en una cepa resistente al linezolid una amplificación del gen ARNr 16S usando los cebadores 27F (5'- AGA GTT TGA TCM TGG CTC AG -3') y 907R (5'- CCG TCA ATT CMT TTR AGT TT -3')[11]. Posteriormente se realizó secuenciación de dicho gen con los cebadores 27F y 519R (5'- GWA TTA CCG CGG CKG CTG -3')[11] y se consultaron las bases de datos del Genbank y del *Ribosomal Database Project*.

Pruebas de sensibilidad antimicrobianas

El estudio de sensibilidad inicial se realizó con la tarjeta Vitek® 559 (HSLL) o por disco-difusión (HUSD), según las recomendaciones del *Clinical and Laboratory Standards Institute* (CLSI)[12]. Posteriormente, se determinó la concentración mínima inhibitoria (CMI) de los aislados de *S. hominis* resistentes al linezolid mediante tiras de E-test® (bioMérieux, Francia), para los siguientes antimicrobianos: linezolid, oxacilina, vancomicina, teicoplanina, ciprofloxacino, eritromicina, clindamicina, trimetoprim-sulfametoxazol (cotrimoxazol), y daptomicina. Se consideraron los puntos de corte definidos por el CLSI[12].

Detección de mutaciones en ARNr 23S

Para identificar las posibles mutaciones, amplificamos el dominio V del gen ARNr 23S en tres aislados de *S. hominis* sensibles al linezolid (todos fueron detectados en hemocultivos, uno del HSLL y dos del HUSD), y en cinco aislados de *S. hominis* resistentes al linezolid (tres del HSLL y dos del HUSD). Se usaron los cebadores 5'-TGG GCA CTG TCT CAA CGA-3' (correspondientes a las bases 1984-2001 del ARN 23S de *E. coli*) y 5'-GGA TAG GGA CCG AAC TGT CTC-3' (correspondientes a las bases 2597-2617 del ARN 23S de *E. coli*) para amplificar un fragmento de 634 pb[8]. Los productos de PCR

fueron secuenciados y alineados con las correspondientes secuencias de oligonucleótidos de la cepa de referencia de *Staphylococcus aureus* (número de acceso en GenBank X68425).

Detección del gen cfr

Se realizó una PCR para detectar la presencia del gen *cfr*, según las condiciones descritas por Kehrenberg[13]. Como control positivo para esta PCR, se usó una cepa de *S. aureus* resistente al linezolid y a la meticilina procedente del Hospital Clínico San Carlos, Madrid[14].

Estudios de epidemiología molecular

La relación clonal de los aislados de *S. hominis* resistentes al linezolid se determinó mediante electroforesis en campo pulsado (ECP). En cada ECP, se incluyeron algunos aislados de *S. hominis* sensibles al linezolid. Al principio del estudio, se usó *Sma*I como enzima de restricción, pero sólo se visualizaron 3-5 bandas para cada cepa. Luego, en una segunda fase, se empleó la enzima de restricción ApaI, según el protocolo descrito por Sorlozano y Vindel[15]. Los fragmentos de ADN cromosómicos se separaron usando el sistema Chef-DR III® (Bio-Rad, Richmond, EE.UU.), con las siguientes condiciones: pulso inicial 0,1 s, pulso final 30 s, tiempo 24 h a 6 V/cm. Los patrones de bandas de la ECP se interpretaron según los criterios de Tenover[16].

Resultados

Durante el periodo del estudio (marzo 2008-febrero 2009), se identificaron en los dos hospitales un total de 15 aislados clínicos de *S. hominis* subsp. *hominis* resistentes al linezolid en 14 pacientes distintos (tabla 1). Un paciente tuvo dos hemocultivos positivos a este microorganismo (con el mismo antibiograma) en dos ingresos diferentes separados por 100 días. De los 15 aislados, 12 se detectaron en pacientes ingresados en el HSLL (11 de ellos en la UCI) y los otros 3 en el HUSD (todos ellos en la Subunidad Quirúrgica-Traumatológica de la UCI). La evolución en los 15 episodios infecciosos fue buena en 12 de ellos, otros 2 pacientes fallecieron por causas no relacionadas, mientras que el paciente restante desarrolló una bacteriemia con infección del cable de marcapasos por este microorganismo, falleciendo a los pocos días (tabla 1).

Los aislados se obtuvieron principalmente de hemocultivos (9), seguido de catéteres (2), y líquidos estériles (2). Además, se llevó a cabo un estudio de colonización (nasal, axilar, e inguinal) en los últimos tres pacientes ingresados en HSLL. Todos ellos tuvieron colonización axilar e inguinal por *S. hominis* multirresistente, con el mismo antibiotipo que su cepa clínica correspondiente.

La secuenciación del gen ARNr 16S confirmó la identificación de *S. hominis* subsp. *hominis*. Todos estos aislados fueron resistentes a la penicilina, oxacilina (CMI > 256 μg/ml), teicoplanina (CMI > 256 μg/ml), trimetoprim-sulfametoxazol (CMI > 32 μg/ml), ciprofloxacino (CMI > 32 μg/ml), levofloxacino, tobramicina y linezolid (CMI ≥ 96 μg/ml). Todos estos aislados fueron sensibles a la eritromicina, tetraciclina, gentamicina y daptomicina. La CMI de la daptomicina fue de 0,25 μg/ml en todos ellos. Respecto a la vancomicina, todas las cepas presentaron una CMI de 4 μg/ml, todavía sensibles según las recomendaciones del CLSI[12] (S ≤ 4 μg/ml, I 8-16 μg/ml, R >32 μg/ml) pero resistentes según los criterios del *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) de enero de 2011[17] (S ≤ 2 μg/ml, R >2 μg/ml).

En relación con la clindamicina, casi todos los aislados tuvieron una sensibilidad intermedia a este antibiótico (CMI = 0,75 μg/ml), excepto en dos aislados. Uno de ellos fue sensible (CMI = 0,5 μg/ml) y el otro resistente a la clindamicina (CMI = 4 μg/ml). Todas las

Tabla 1
Características de los pacientes con *Staphylococcus hominis* resistente al linezolid

Paciente	Hospital	Fecha de aislamiento	Muestra	Sexo	Días de tratamiento con linezolid previos al aislamiento	Tratamiento previo con glucopéptidos	Probable origen contaminante	Tratamiento dirigido	Evolución
1	HSLL	29/03/2008	Hemocultivo	F	16	No	Sí	No	Muerte no relacionada
2	HSLL	22/05/2008	Hemocultivo	F	10	Vancomicina	No	Vancomicina	Buena
3	HSLL	06/08/2008	Hemocultivo	F	0	No	Sí	No	Buena
4	HSLL	19/08/2008	Hemocultivo	M	9	Vancomicina	Sí	No	Buena
5	HSLL	01/09/2008	Hemocultivo, cable marcapasos	F	6	No	No	Vancomicina	Muerte relacionada
6	HSLL	01/10/2008	Hemocultivo	M	0	No	Sí	Vancomicina	Buena
6	HSLL	21/01/2009	Hemocultivo	M	4	Vancomicina	No	Vancomicina	Buena
7	HUSD	11/11/2008	LCR	F	19	Vancomicina	Sí	Vancomicina	Muerte no relacionada
8	HUSD	16/11/2008	Catéter	M	17	No	No	Vancomicina	Buena
9	HSLL	17/12/2008	Hemocultivo	M	3	No	Sí	No	Buena
10	HSLL	28/01/2009	Líquido sinovial	F	42	No	Sí	No	Buena
11	HSLL	06/02/2009	Exudado herida	M	1	No	No	Vancomicina	Buena
12	HSLL	13/02/2009	Hemocultivo	M	0	No	Sí	No	Buena
13	HSLL	23/02/2009	BAS	F	11	Vancomicina	No	Tigeciclina	Buena
14	HUSD	26/02/2009	Catéter	M	11	No	No	Tigeciclina	Buena

HSLL: Hospital Son Llàtzer; HUSD: Hospital Universitario Son Dureta; LCR: líquido cefalorraquídeo; BAS: aspirado bronquial.

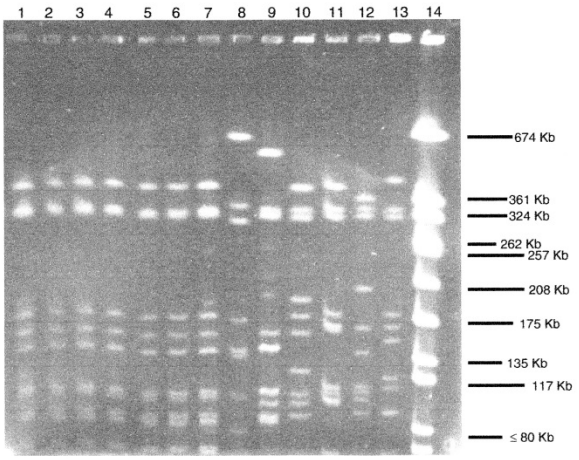

Figura 1. Electroforesis en campo pulsado de trece aislados de *Staphylococcus hominis* utilizando *Apa*I como enzima de restricción. Líneas 1-6: aislados de *S. hominis* resistentes al linezolid del Hospital Son Llàtzer (HSLL). Línea 7: aislado de *S. hominis* resistente al linezolid del Hospital Universitario Son Dureta (HUSD). Línea 8: aislado de *S. hominis* sensible al linezolid del HUSD. Líneas 9-13: aislados de *S. hominis* sensibles al linezolid del HSLL. Línea 14: cepa de referencia de *Staphylococcus aureus* NCTC 8325.

cepas fueron sensibles a la rifampicina, excepto una que fue intermedia.

Los cinco aislados de *S. hominis* resistentes al linezolid secuenciados tuvieron la mutación G2576T (según numeración de *E. coli*) en el dominio V del gen ARNr 23S, mientras que ninguno de los tres aislados de *S. hominis* sensibles al linezolid secuenciados presentaron dicha mutación. En comparación con la secuencia del dominio V del gen ARNr 23S de la cepa control de *S. aureus* (número acceso GenBank X68425), nosotros también encontramos en todos los ocho aislados de *S. hominis* (tanto sensibles como resistentes al linezolid) la sustitución C2163T. Por ello, consideramos que esta sustitución es un polimorfismo de especie. No se detectó el gen *cfr* en ninguno de los aislados de *S. hominis*.

La ECP, tras digestión con *Sma*I, mostró que todos los aislados de *S. hominis* resistentes al linezolid tenían el mismo patrón electroforético, aunque mostraron sólo tres bandas, mientras que los aislados sensibles tuvieron 3-4 bandas en diferentes posiciones. La ECP usando ApaI como enzima de restricción mostró que todos los aislados resistentes al linezolid pertenecían al mismo clon, mientras que todos los aislados de *S. hominis* sensibles pertenecían a diferentes clones (fig. 1).

Discusión

El linezolid fue aprobado para uso clínico en Estados Unidos en el año 2000 y en Europa en el 2001, con una excelente actividad contra la mayoría de los cocos grampositivos[7]. No se encontraron aislados de cocos grampositivos resistentes al linezolid antes de su aprobación y además, la resistencia por mutación es difícil de seleccionar *in vitro*[18]. No obstante, en el año 2001, un año después de su comercialización, se detectó en Boston, EE.UU., el primer aislado clínico de estafilococo resistente al linezolid. Este primer caso estaba producido por una cepa de *S. aureus* resistente a la meticilina (SARM) con la mutación G2576T en el gen ARNr 23S, que se había detectado en un paciente que había recibido diálisis peritoneal en tratamiento previo con linezolid[8]. El primer SCN resistente al linezolid publicado fue una cepa de *S. epidermidis* detectada en EE.UU. en el 2002, también con la misma mutación[19]. Los primeros dos brotes descritos de estafilococos resistentes al linezolid ocurrieron en el 2005, uno de ellos en Pittsburgh, EE.UU.[20], y el otro en Dublín, Irlanda[21]; ambos producidos por dos cepas de *S. epidermidis*. El mecanismo de resistencia al linezolid en los aislados de *S. epidermidis* del hospital americano no es conocido, pero todas las cepas resistentes del hospital irlandés contenían la mutación G2576T. En España, se han descrito varios brotes de SCN resistentes al linezolid con la mutación G2576T en aislados de *S. epidermidis*[22], *Staphylococcus haemolyticus*[23] y *S. hominis*[15]. Por lo que respecta al gen *cfr*, la primera cepa de *S. aureus* detectada en humanos con dicho gen ocurrió en 2005 en Colombia[24], mientras que el primer SCN con el gen *cfr* se aisló en 2007 en EE.UU. en una cepa de *S. epidermidis*[25]. Asimismo, en 2008 se detectó el primer brote de SARM con el gen *cfr* en el Hospital Clínico San Carlos, Madrid[14].

En marzo de 2008, se detectó la primera cepa de *S. hominis* resistente al linezolid en Mallorca, en una paciente ingresada en la UCI del HSLL, que comenzó tratamiento con linezolid 16 días antes del aislamiento de la cepa resistente. El segundo aislado se observó en mayo 2008, en otra paciente de la misma UCI también tratada con linezolid. Pero, en agosto del 2008, a pesar de las medidas de control implantadas (aislamiento de los pacientes infectados y administración de un tratamiento antibiótico efectivo) se estableció en la UCI del HSLL una situación endémica, que persiste actualmente. Además, en enero de 2009, se detectó en el HSLL el primer paciente infectado por esta cepa hospitalizado fuera de la UCI. En el HUSD, los aislados de *S. hominis* resistentes al linezolid se detectaron por primera vez en dos pacientes ingresados en la UCI de este hospital en noviembre del 2008. Posteriormente, se aisló de nuevo en febrero del 2009 también en una paciente ingresada en la UCI.

De modo similar al primer brote descrito de *S. hominis* resistente al linezolid en Granada, España[15], en la mayoría de nuestros pacientes, este microorganismo se aisló solamente en un vial de hemocultivo de un total de cuatro, por lo que podría considerarse como un contaminante de los hemocultivos. Pero, en algunos casos, detectamos *S. hominis* resistente al linezolid en líquidos corporales, aspirados bronquiales, o simultáneamente en catéter y hemocultivos. Llama la atención que algunos de los pacientes infectados tenían además una colonización axilar e inguinal por la misma cepa,

pero no colonización nasal. Esto está en concordancia con el trabajo de Center et al en el que encontraron, en neonatos, una mayor detección de aislados de *Staphylococcus warneri* con sensibilidad disminuida a la vancomicina en muestras cutáneas y de heces (32 aislados en 39 muestras) que en nasofaringe (7 de 39)[26].

Nosotros encontramos la mutación G2576T en el gen ARNr 23S en todos los cinco aislados de *S. hominis* resistentes al linezolid que secuenciamos. La aparición de la resistencia al linezolid se desarrolla en un proceso de dos pasos: inicialmente tiene lugar una mutación en la posición 2576 de una de las copias del gen ARNr 23S (los estafilococos poseen cinco o seis copias de este gen), seguida de una recombinación intracromosómica (conversión de genes), en la que se distribuye dicha mutación a las otras copias de los genes, confiriendo resistencia al linezolid[9]. Los aislados de estafilococos con un mayor número de copias mutadas del gen ARNr 23S suelen tener unos valores de la CMI del linezolid más altos[27].

Los mecanismos de resistencia al linezolid en *S. hominis* se caracterizaron por primera vez en los aislados del brote de Granada[15]. Ese trabajo describió la presencia de dos «nuevas mutaciones» simultáneamente en aislados de *S. hominis* resistentes al linezolid: C2190T y G2603T. Sin embargo, ellos no usaron la numeración de *E. coli* para las mutaciones, sino la numeración de la cepa de referencia de *S. aureus* X68425. De hecho, su mutación G2603T corresponde realmente a la clásica G2576T, como encontramos en nuestro estudio. Además, en el estudio de Granada no secuenciaron aislados de *S. hominis* sensibles al linezolid, con lo que la mutación C2190T que encontraron en los aislados resistentes (C2163T con la numeración de *E. coli*) es de hecho un polimorfismo de la especie *S. hominis*, también presente uniformemente en los aislados sensibles al linezolid de esta especie. No se ha estudiado específicamente si este polimorfismo C2163T confiere alguna ventaja a *S. hominis* en relación a otras especies de SCN, si bien, al detectarse la resistencia al linezolid en diferentes especies de estafilococos sin este polimorfismo, es probable que éste no juegue ningún papel en la resistencia al linezolid. Recientemente, se ha publicado la detección de la mutación G2576T en cinco aislados de *S. hominis* resistentes al linezolid en pacientes ingresados en la UCI de dos hospitales de Sicilia[28].

Dos aspectos adicionales no quedan claros en nuestro brote. Uno de ellos es la diseminación de la cepa desde la UCI del HSLL a la UCI del HUSD. En 2008, no se produjo ninguna transferencia de pacientes entre las UCI de ambos hospitales y tampoco hubo ningún traslado de instrumental entre ellas. No obstante, dos enfermeras estaban trabajando en las dos UCI al mismo tiempo, aunque no se realizó estudio de colonización del personal sanitario. De modo similar, se desconocen los factores responsables de la persistencia de la cepa en la UCI del HSLL, produciendo 1-2 infecciones cada mes. La cepa podría transmitirse de paciente a paciente a través de las manos del personal sanitario colonizado[29], o bien, podría haber un reservorio en el ambiente de la UCI. En el brote irlandés de *S. epidermidis* resistente al linezolid, los autores encontraron la misma cepa resistente en las cercanías de los pacientes colonizados y en un ordenador usado por el personal del la UCI[21].

Una larga duración del tratamiento con linezolid, una dosificación insuficiente, y la administración repetida del linezolid son factores de riesgo para desarrollar resistencia a este antimicrobiano[30]. La resistencia al linezolid no sólo se ha observado en pacientes con un tratamiento prolongado a dicho antimicrobiano, sino también en casos sin exposición obvia[20–23]. En nuestra serie, la mayoría de pacientes infectados (12 de 15 episodios infecciosos, el 80,0%) habían recibido ciclos de linezolid antes de la detección del aislado resistente. Con todo, 3 pacientes (20,0%) no recibieron linezolid, lo que sugiere la adquisición por transmisión cruzada[21,23].

Todos los aislados de *S. hominis* resistentes al linezolid fueron también resistentes a la teicoplanina y tuvieron por E-test CMI a la vancomicina de 4,0 μg/ml. En el momento actual, 4,0 μg/ml es el límite superior del rango de sensibilidad según el CLSI[12], y resistente según el EUCAST[17]. Este perfil de resistencia a glucopéptidos está también presente en aislados resistentes al linezolid de *S. epidermidis*[20], *S. haemolyticus*[23] y *S. hominis*[15,28], todos ellas con la mutación G2576T. La relación entre esta mutación en el gen ARNr 23S y la resistencia a los glucopéptidos es desconocida. La resistencia a los glucopéptidos en *Staphylococcus* spp. es multifactorial[31], siendo el engrosamiento de la pared celular una característica común[32].

La incidencia creciente de infecciones causadas por aislados de estafilococos multirresistentes, incluyendo resistencia a la teicoplanina y al linezolid, es preocupante. Es crucial hacer un uso racional del linezolid y mantener un control activo de dicha resistencia para preservar la utilidad clínica de este antimicrobiano.

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Conflicto de intereses

Los autores declaran no tener conflicto de intereses.

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Transferable Multidrug Resistance Plasmid Carrying *cfr* Associated with *tet*(L), *ant(4′)-Ia*, and *dfrK* Genes from a Clinical Methicillin-Resistant *Staphylococcus aureus* ST125 Strain

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A multidrug resistance (MDR) conjugative plasmid of ca. 50 kb (designated pERGB) was detected in a linezolid and methicillin-resistant *Staphylococcus aureus* strain with sequence type 125 (ST125-MRSA-IVc). This strain was detected in two patients with chronic obstructive pulmonary disease, previously treated with multiple antimicrobials, including linezolid. pERGB was transferable by conjugation and carried the resistance genes *cfr* (oxazolidinones, phenicols, lincosamides, pleuromutilins, and streptogramin A), *ant(4′)-Ia* (tobramycin), *tet*(L) (tetracycline), and *dfrK* (trimethoprim). A novel genetic structure, linking all of these resistance genes for the first time, was elucidated through sequencing of a 15,259-bp fragment from pERGB. Active surveillance to prevent the dissemination of such highly concerning MDR transferable elements is needed.

Linezolid was the first oxazolidinone approved for the treatment of Gram-positive bacteria and is still the only antimicrobial in this class released for clinical use. It has been commercialized in the United States since 2000 (2) and in Spain since 2002 (3). Linezolid resistance in staphylococci is very uncommon, although it has increased in the recent years.

The most frequently reported mechanism of linezolid resistance in staphylococci is a point mutation within the central loop of domain V of the 23S rRNA gene (9), followed by mutation in the ribosomal protein L3 or L4 (17). Both forms of resistance are mainly caused by prolonged exposure to linezolid and are not transferable between strains. A third mechanism of resistance is determined by the *cfr* gene, encoding an rRNA methyltransferase that methylates the adenosine at position A2503 (*Escherichia coli* numbering) in the 23S rRNA (13). This enzyme confers cross-resistance to linezolid and four other classes of antimicrobial agents: phenicols, lincosamides, pleuromutilins, and streptogramin A. The *cfr* gene is often located in a plasmid which may be horizontally transferred (18). It was originally identified on the pSCSF1 plasmid from a *Staphylococcus sciuri* isolate obtained from the nasal swab of a florfenicol-treated calf with a respiratory infection in 1997 (26). In humans, the first clinical isolate documented to carry the *cfr* gene was a methicillin-resistant *Staphylococcus aureus* (MRSA) strain isolated from a patient with respiratory infection in Colombia in 2005 (29). The first outbreak by MRSA carrying the *cfr* gene was reported in 2008 and involved 15 patients admitted in the intensive care unit of a hospital from Madrid, Spain (20).

Here we describe two cases of respiratory infection by *cfr*-positive MRSA isolates detected in Majorca, Spain, occurring in patients with chronic obstructive pulmonary disease (COPD) admitted in August 2010 in the same unit of the main tertiary hospital of the island. Moreover, we show that *cfr* was located in a novel transferable plasmid together with multiple other antibiotic resistance determinants.

The linezolid-resistant MRSA isolates from the two patients had the same disk diffusion susceptibility profile. According to Clinical and Laboratory Standards Institute (CLSI) (4) breakpoints, both isolates were resistant to penicillin, oxacillin, cefoxitin, ciprofloxacin, tobramycin, clindamycin, chloramphenicol, and tetracycline and susceptible to erythromycin, gentamicin, rifampin, and teicoplanin. The MIC of vancomycin was determined by Etest (bioMérieux, France) to be 2 μg/ml, indicating susceptibility according to CLSI. Regarding linezolid and trimethoprim-sulfamethoxazole, the isolates were apparently susceptible by disk diffusion, but a double halo of inhibition was noted in both cases. When the isolates were tested by Etest, a double halo of inhibition was again visualized, with a MIC for linezolid of 6 to 8 μg/ml after 24 h of incubation; thus, it was considered resistant. The MIC for trimethoprim-sulfamethoxazole determined by Etest was 1.5 μg/ml (still within the susceptibility range).

Interestingly, a linezolid-susceptible MRSA isolate was previously identified in sputum cultures from both patients (in June 2009 [patient 1] and February 2009 [patient 2]), and both received linezolid therapy at that time. Moreover, patient 1 was seen 32 times for respiratory infections from October 2006 to August 2010, receiving in every admission antimicrobial therapy, including several linezolid courses. The resistance profiles of the two linezolid-susceptible and two linezolid-resistant MRSA isolates are shown in Table 1.

Clonal relatedness of the previous linezolid-susceptible and subsequent linezolid-resistant MRSA isolates from both patients was evaluated by pulsed-field gel electrophoresis (PFGE) (28) and multilocus sequence typing (MLST) (5). Typing of the staphylococcal cassette chromosome *mec* (SCC*mec*) was performed with three PCR techniques: a multiplex PCR assay for the cassette chromosome recombinase (*ccr*) genes (7), another for the class B *mec* gene complex (16), and a multiplex PCR assay for the subtyping of SCC*mec* IV (19). A linezolid-resistant *cfr*-positive MRSA isolate

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TABLE 1 Resistance profiles and genes detected in linezolid-susceptible and -resistant MRSA clinical isolates from patients 1 and 2 and in the transconjugant derivative

Patient or description	Date	Isolate no.	Sequence type	Resistance phenotype[a]	Presence of gene			
					ant(4')-Ia (*aadD*)	*cfr*	*tet*(L)	*dfrK*
1	June 2009	50115	ST125-IVc	OXA, CIP, TOB	+	−	−	−
	August 2010	69371	ST125-IVc	OXA, CIP, TOB, CLI, LIN, CLO, TET, TS	+	+	+	+
2	February 2009	44207	ST22-IVh	OXA, CIP, ERY, iCLI	−	−	−	−
	August 2010	69533	ST125-IVc	OXA, CIP, TOB, CLI, LIN, CLO, TET, TS	+	+	+	+
Transconjugant				TOB, CLI, LIN, CLO, TET, TS, RIF	+	+	+	+

[a] OXA, oxacillin; CIP, ciprofloxacin; TOB, tobramycin; ERY, erythromycin; CLI, clindamycin; iCLI, inducible clindamycin resistance (erythromycin-clindamycin D-zone test positive); LIN, linezolid; CLO, chloramphenicol; TET, tetracycline; TS, trimethoprim-sulfamethoxazole (cotrimoxazole); RIF, rifampin.

(HC08 strain) from the outbreak reported in Madrid (20) was included for comparative purposes. The linezolid-resistant isolates from both patients and interestingly also the first linezolid-susceptible isolate from patient 1 had indistinguishable electrophoretic band patterns, which corresponded to the ST125-MRSA-IVc clone, one of two MRSA clones predominant in Majorca (1, 24) and also the most prevalent MRSA clone in Spain since 1998 (30). On the other hand, the linezolid-susceptible isolate from patient 2 belonged to ST22-MRSA-IVh (EMRSA-15 clone), also prevalent in Majorca, whereas the HC08 strain corresponded to ST228-MRSA-I.

To determine the mechanism of resistance to linezolid, a PCR assay to detect the presence of the *cfr* gene (14) was performed with genomic DNA extracted using the QIAamp DNA minikit (Qiagen, Germany). The HC08 strain (20) was used as a positive control. The PCR assay for the *cfr* gene was negative for the linezolid-susceptible MRSA isolates and positive for those showing resistance to the drug (Table 1).

Conjugation experiments were then performed to assess whether the *cfr* gene was located on a transferable plasmid, adapting the protocol described by Shore et al. (27). The linezolid-resistant MRSA isolate from patient 1 was used as a donor, and a rifampin-resistant mutant of ATCC 29213 *S. aureus* strain was used as a recipient. The transconjugants were selected in brain heart infusion (BHI) agar plates with rifampin (20 μg/ml) and chloramphenicol (15 μg/ml) and checked through the analysis of plasmid and susceptibility profiles, *cfr* PCR assay (14), and PFGE.

The antimicrobial susceptibility profiles of linezolid-resistant MRSA from patient 1 (donor), rifampin-resistant ATCC 29213 *S. aureus* (recipient), and a selected transconjugant for several antibiotics were determined by Etest and/or broth microdilution, and results are presented in Table 2. Remarkably, the transconjugant had acquired resistance or reduced susceptibility to clindamycin, chloramphenicol, and linezolid (as expected for *cfr* transfer) but also to tetracycline, tobramycin, and cotrimoxazole. The tigecycline MIC was also slightly enhanced.

The PCR assay for the *cfr* gene in the plasmid DNA (14) extracted from the transconjugant using the Qiagen Plasmid Midi kit was also positive. The size of the plasmid was determined through analysis of the EcoRI restriction fragments, with a result of ca. 50 kb. The plasmid was larger than the two first *cfr* plasmids characterized, both detected in animals, plasmids pSCFS1 (17.1 kb) (12) and pSCFS3 (35.7 kb) (14), but the size was similar to that of other *cfr* plasmids: pSCFS6 (43 kb, also detected in animals) (15) and pSCFS7 (45 kb, detected in humans) (27).

For the detection of the additional resistance genes transferred,

TABLE 2 Antimicrobial resistance profiles of isolate 69371 (*cfr* positive) from patient 1 (donor), a rifampin-resistant (Rif^r) ATCC 29213 *S. aureus* strain (recipient), and its transconjugant derivative

Antimicrobial	Susceptibility testing method	MIC (μg/ml) for strain		
		69371 (donor)	ATCC 29213 Rif^r (recipient)	Transconjugant
Penicillin	Etest	>32	0.5	0.38
Oxacillin	Etest	>256	0.5	0.5
Erythromycin	Etest	0.19	0.094	0.064
Clindamycin	Etest	>256	0.047	>256
Ciprofloxacin	Etest	>32	0.19	0.19
Trimethoprim-sulfamethoxazole (1/19)[a]	Etest	1.5	0.064	0.75
Vancomycin	Etest	2	2	2
Teicoplanin	Etest	1	1	1
Daptomycin	Etest	0.19	0.25	0.25
Tigecycline	Etest	1.5	0.19	0.5
Tetracycline	Etest	16	0.094	16
Linezolid	Etest	6	0.38	3
Tobramycin	Etest	6	0.38	3
Gentamicin	Broth microdilution	0.5	0.25	0.25
Chloramphenicol	Broth microdilution	8	2	8
Rifampin	Broth microdilution	≤0.06	>32	>32

[a] The value on the MIC scale refers to the first component of the combination.

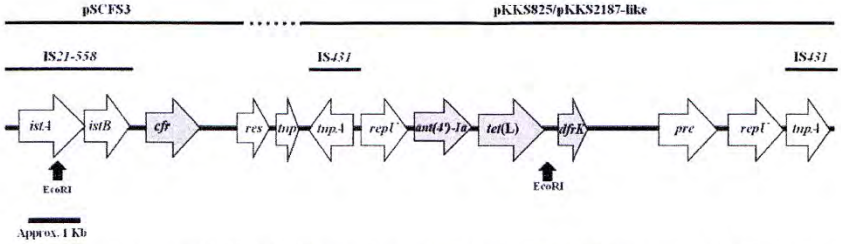

FIG 1 Genetic structure of the sequenced 15,259-bp DNA fragment from the MDR plasmid pERGB. EcoRI restriction sites delimiting the cloned 8,675-bp fragment are represented with black arrows. Regions homologous to sequence of previously described plasmids are indicated in the upper part. The gray bar on the first *repU* gene represents its truncation by the IS*431* 5′ end.

PCR analysis was carried out with the plasmid DNA extract of the transconjugant and clinical isolates, and results are shown in Table 1. The PCR assays for the *ant(4′)-Ia* gene (also known as *aadD*) which confers resistance to tobramycin, amikacin, kanamycin, and neomycin (25), for the *tet*(L) gene (tetracycline resistance) (22), and for the *dfrK* gene (6) (trimethoprim resistance) were positive for the transconjugant derivative and for the two linezolid-resistant MRSA isolates (Table 1). On the other hand, the PCR assays were negative in all cases for the *tet*(K) and *tet*(M) genes (tetracycline resistance) (8), the *vga*(C) gene (pleuromutilin/lincosamide/streptogramin A resistance) (6), and the *fexA* gene (which codifies an efflux protein of phenicols) (14).

To determine the proximity of the *cfr* determinant to the other resistance genes, DNA fragments resulting from EcoRI digestion from the transconjugant derivative were cloned into pUCP24 (21). The resulting plasmids were then transformed into the $CaCl_2$-competent *Escherichia coli* XL1 Blue strain. Transformants were selected on Luria-Bertani (LB) agar plates supplemented with 100 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)–200 μg/ml isopropyl-β-D-thiogalactopyranoside (IPTG) and 5 μg/ml gentamicin. Plasmid DNA, purified from several transformants with the Qiagen plasmid minikit, was screened for *cfr* presence through PCR assays (14), yielding a positive result in one of them. The cloned 8,675-bp EcoRI fragment was fully sequenced through primer walking and contained the resistance genes *cfr*, *ant(4′)-Ia*, and *tet*(L) (Fig. 1). Left and right ends were extended to obtain a 15,259-bp fragment through diverse PCR assays, followed by sequencing, based on available GenBank records: plasmid pSCFS3 (accession number AM086211.1) (14) for the left end and pKKS825 (accession number FN377602.2) (11) and pKKS2187 (accession number FM207105.1) (12) for the right end.

The initial segment of the sequenced fragment corresponded to an entire copy of insertion sequence IS*21-558*, with a homology of 100% with pSCFS3 (14), followed by the *cfr* gene and a noncoding region of 688 bp, also identical to sequence of pSCFS3. Nevertheless, adjacent to this noncoding region, a novel structure was detected, including a putative resolvase (72% nucleotide sequence identity with the *res* gene from *Bacillus megaterium*, GenBank accession number CP003018.1) and a putative transposase (amino acid identity of 63% with a transposase of *Enterococcus faecium*, accession number ZP_03980121.1). Next, an IS*431*-flanked genetic structure was found; an inverted copy of insertion sequence IS*431* was followed by a truncated *repU* gene, with 99% identity to the nucleotide sequence of plasmid pUB110 (23) and very similar to that previously described in pKKS825/pKKS2187 plasmids (10, 11). Downstream, the *ant(4′)-Ia*, *tet*(L), and *dfrK* resistance genes were identified, with sequences nearly identical to those described for the pKKS825 plasmid (11). Further downstream of *dfrK*, two more open reading frames (ORFs) were detected: the *pre*/*mob* gene (97% identical to pKKS2187) and an entire copy of the *repU* gene. At the end of the sequenced fragment, a direct copy of IS*431* was documented (Fig. 1).

In summary, we describe a new plasmid (designated pERGB) that combines several relevant antibiotic resistance determinants, leading to a MDR transferable element that is highly concerning, particularly when acquired by MRSA strains as described in this work. Thus, active surveillance to prevent the dissemination of such MDR elements and strains is urgently needed.

Nucleotide sequence accession number. The described 15,259-bp sequence of plasmid pERGB from MRSA strain 69371 has been deposited in the GenBank database under accession number JN970906.

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Clinical significance of methicillin-resistant *Staphylococcus aureus* colonization in residents in community long-term-care facilities in Spain

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) is highly prevalent in Spanish hospitals and community long-term-care facilities (LTCFs). This longitudinal study was performed in community LTCFs to determine whether MRSA colonization is associated with MRSA infections and overall mortality. Nasal and decubitus ulcer cultures were performed every 6 months for an 18-month period on 178 MRSA-colonized residents (86 490 patient-days) and 196 non-MRSA carriers (97 470 patient-days). Fourteen residents developed MRSA infections and 10 of these were skin and soft tissue infections. Two patients with respiratory infections required hospitalization. The incidence rate of MRSA infection was 0·12/1000 patient-days in MRSA carriers and 0·05/1000 patient-days in non-carriers ($P=0·46$). No difference in MRSA infection rate was found according to the duration of MRSA colonization ($P=0·69$). The mortality rate was 20·8% in colonized residents and 16·8% in non-carriers; four residents with MRSA infection died. Overall mortality was statistically similar in both cohorts. Our results suggest that despite a high prevalence of MRSA colonization in LTCFs, MRSA infections are neither frequent nor severe while colonized residents remain at the facility. The epidemiological impact of an MRSA reservoir is more relevant than the clinical impact of this colonization for an individual resident and supports current recommendations to control MRSA spread in community LTCFs.

Key words: Epidemiology, geriatrics, long-term care, MRSA, MRSA infections, multiresistant microorganism, nursing homes, *S. aureus*.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has a high prevalence in acute-care hospitals in Spain, and also in long-term-care facilities (LTCFs) [1–3]. This scenario is similar to other countries of the European Union [4–8]. In the nosocomial setting patients with persistent MRSA carriage have a higher risk of developing MRSA infections [9, 10] than methicillin-susceptible *S. aureus* (MSSA) carriers and non-carriers. Although, it appears that MRSA colonization in LTC settings might have different clinical implications than in acute-care hospitals, few studies

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have addressed this issue and most report a low prevalence of MRSA infection in residents [11, 12]. A relatively small number of residents require hospitalization or die as a consequence of MRSA infections and this suggests that severe infections are uncommon in this population. The most frequent MRSA infections in LTCFs are skin and soft tissue infections while bloodstream infections account for about 10% of cases [13].

Very few longitudinal studies have investigated the incidence of MRSA infections in residents in LTCFs [12, 14]. We therefore considered it necessary to analyse the clinical impact of MRSA colonization in this population in order to identify suitable measures to prevent spread and infections due to MRSA in this setting. To this end a multicentre longitudinal study was performed among residents in community LTCFs to determine the incidence of MRSA infection and assess whether MRSA colonization is associated with greater risk of infection and overall mortality.

METHODS

Study population and characteristics of community LTCFs

Characteristics of this population have been described previously [2]. Nine community LTCFs for the elderly, located in two communities in Spain (Catalonia and Balearic Islands) with 1586 beds (median 120, range 72–552) were included. Five were located in the catchment area of a 900-bed acute-care hospital (Hospital Universitari de Bellvitge), three in that of a 490-bed hospital (Corporació Sanitària Parc Taulí) and one in that of an 800-bed hospital (Hospital Universitari Son Dureta). These facilities provide care for the elderly long-term resident, who may be disabled or infirm. Each LTCF has a dementia ward and its own medical staff. Residents are accommodated in rooms with up to three beds. Surveillance for MRSA and decolonization procedures are not routine. In addition to standard precautions for all patient care, contact precautions are applied for residents colonized or infected by MRSA. Known MRSA carriers are not denied admission [15].

Study design

This was a multicentre prospective cohort study conducted from November 2005 to May 2007. The studied population consisted of all residents in the LTCFs at baseline ($n=1377$) and was identical to that of our previously published cross-sectional study [2]. A total of 231 residents was found to be colonized with MRSA at baseline (MRSA carriers cohort). A representative sample of non-MRSA carriers was selected from the 1146 residents without MRSA at baseline as follows: for each MRSA carrier identified, one non-MRSA carrier was randomly selected from the same ward and screened 6 months later. Those with two consecutive negative cultures were included in the non-MRSA carrier cohort ($n=196$). Subjects were visited by the investigators every 6 months over an 18-month period.

During this period no changes were made to infection control practices in the LTCFs and data from the study results were not available to clinical staff. Decolonization treatment or contact precautions were not applied to the MRSA carriers detected throughout the study.

Data collection and definitions

Complete clinical data were obtained for all residents at baseline and medical charts were reviewed thereafter at 6-monthly intervals. Occurrences of infections, decubitus ulcers, antibiotic use, hospital admission and deaths were recorded. In the MRSA carrier cohort, persistent colonization was defined as at least two MRSA-positive cultures separated by fewer than two negative cultures. Transient colonization was defined as two or more negative cultures after a single positive culture for MRSA [16]. Duration of carriage was defined as the period from the first positive culture until the first negative culture with a consecutive negative culture if available and only residents who survived at the end of the study were considered for this analysis. MRSA infection was recorded in the clinical charts of each facility.

Microbiological methods

Nasal, and where applicable, decubitus ulcer swabs were obtained for culture every 6 months. Swabs were placed in Stuart's transport medium and plated on coagulase-mannitol agar plates and selective MRSA agar (MRSA Select, Bio-Rad Laboratories, Spain) before inoculating into brain heart infusion plus 7% NaCl. After 24 h of incubation at 35 °C, broths were subcultured on coagulase-mannitol and selective MRSA agar; plates were incubated for 48 h and inspected daily. Putative *S. aureus* colonies were identified by the latex agglutination test (Pastorex®

Table 1. *Characteristics of residents who were lost to follow-up with those followed to completion for the study period*

	Completed follow-up ($n=280$)	Lost to follow-up ($n=147$)	P
Female sex, *n* (%)	191 (68·0)	102 (69·4)	0·08
Age, yr, mean (S.D.)	80·9 (11·6)	83·5 (10·8)	0·06
Charlson Index $\geqslant 2$, *n* (%)	99 (35·4)	70 (47·6)	0·87
Barthel Index <30, *n* (%)	101 (36·2)	75 (51·1)	0·009
Decubitus ulcers, *n* (%)	40 (14·2)	35 (23·8)	0·22
Centre <150 beds *n* (%)	109 (39·1)	60 (40·8)	0·77
Centre MRSA prevalence $>20\%$, *n* (%)	172 (61·6)	88 (59·9)	0·15
MRSA colonization, *n* (%)	**128 (45·6)**	**104 (70·7)**	**0·17**
Two-site colonization, *n* (%)	17 (5·0)	17 (11·6)	0·59
Infections (all microorganisms), *n* (%)	152 (54·1)	45 (30·6)	0·34
MRSA infections, *n* (%)	8 (2·8)	5 (3·4)	0·25
Hospital admissions, *n* (%)	39 (13·9)	20 (13·6)	0·08

Staph-plus, Bio-Rad Laboratories) and DNase production (DNase Test Agar, Biomedics, Spain). Methicillin resistance was determined by the cefoxitin disk diffusion method and antimicrobial susceptibility testing was performed by disk diffusion following Clinical and Laboratory Standards Institute recommendations [17].

Statistical analysis

Secondary outcomes (MRSA infections and overall mortality) were compared between prevalent MRSA carriers and non-MRSA carriers. Categorical variables were analysed with χ^2 or Fisher's exact tests as appropriate and continuous variables by Student's *t* test or non-parametric tests. Mortality was compared by the Kaplan–Meier method. All statistical tests were two-tailed and $P<0{\cdot}05$ was deemed significant. SPSS package version 15·0 was used (SPSS Inc., USA).

Approval for the study was obtained from the Research Ethics Committee of the Hospital Universitari de Bellvitge. No written informed consent was obtained because the study met the criteria for a waiver of this requirement.

RESULTS

The MRSA cohort comprised 178 colonized residents (86 490 patient-days) and the non-carrier cohort 196 patients (97 470 patient-days). Over the study period, 147 residents were lost to follow-up (53 at 6 months, 60 at 12 months and 34 at 18 months), 99 residents died and 30 were discharged. Table 1 compares residents who were lost to follow-up with those followed to completion.

Table 2. *Incidence rate of MRSA infection during the 18-month period related to the duration of MRSA colonization*

Duration of MRSA colonization	Follow-up (days)	No. of MRSA infections	Incidence rate of MRSA infections
<6 months	156 60	3	0·19/1000 patient-days
6 months	9720	0	—
12 months	5205	3	0·58/1000 patient-days
18 months	32 202	2	0·06/1000 patient-days

Overall 14 residents developed MRSA infections, nine in the MRSA cohort and five in the non-carrier cohort. The type of infections were: 10 skin and soft tissue infections, seven related to decubitus ulcers, one urinary tract infection, one chronic external otitis and two respiratory infections which both required hospital admission.

The incidence rate of MRSA infection in the total MRSA cohort ($n=178$) was 0·12/1000 patient-days and in the 196 non-carriers, 39 residents acquired MRSA colonization during the study, giving an incidence rate of MRSA infection in this cohort of 0·05/1000 patient-days. The incidence rate of MRSA infection was statistically similar for prevalent MRSA carriers and residents with newly acquired MRSA colonization ($P=0{\cdot}46$). Table 2 shows that no

Table 3. *Comparison of clinical outcomes in the MRSA-colonized cohort and the non-carrier cohort*

	Non-carriers ($N=196$) n (%)	MRSA-colonized cohort ($N=178$) n (%)	RR (95% CI)
MRSA infections	4 (2·1)	10 (4·6)	2·85 (0·88–9·28)
All infections	94 (48·0)	103 (57·9)	1·49 (0·99–2·24)
Hospital admissions	27 (13·8)	32 (18·0)	1·37 (0·79–2·40)

RR, Relative risk; CI, confidence interval.

difference was found in MRSA infection rate between transient and persistent MRSA carriers (lineal regression $P=0·69$). In addition there were no differences in infections of any aetiology for both cohorts, and MRSA carriers did not require more hospital admissions than non-carriers during the study period (Table 3). The mortality rate was 20·8% in residents in the MRSA cohort and 16·8% in non-carriers. Four of 14 residents with MRSA infection died during the study period but these were not attributed to MRSA infection. No statistical difference was found in the overall mortality in either group (log rank test 0·19, $P=0·66$) (Fig. 1).

DISCUSSION

In a previous study we reported a prevalence of MRSA colonization of about 17% in residents of community LTCFs in Spain [2], which represents a large reservoir of this microorganism in the healthcare setting. Several studies have highlighted the relevance of this epidemiological aspect which might influence the infection control practices implemented by acute-care hospitals [18–22] but there are limited data on the relationship of MRSA colonization and the development of infection in residents of LTCFs [12, 14]. This aspect has usually been assessed in settings where patients are at great risk of MRSA infection, such as intensive care units [10]. Our findings show that MRSA carriers in community LTCFs are not at high risk of developing severe MRSA infection while residing at the facility. This is in agreement with the out-of-hospital risk of MRSA infection reported in another population [23]. Moreover, from a clinical point of view, MRSA infections were not severe and only 2/14 cases required hospitalization. As previously reported, we found that the main MRSA infections were skin and soft tissue infections. Remarkably the majority of infections were associated with the presence of decubitus ulcers, the most frequent skin lesion in this population. This emphasizes the need to enhance efforts to prevent the development of decubitus ulcers. Since in community LTCFs accurate microbiological diagnoses are often lacking, MRSA infections could have been underestimated and therefore we analysed the incidence of infection of any aetiology in both cohorts and found no differences. Prior studies have demonstrated an incidence of MRSA infections of 6·5% [13] and a relative risk of 3·6 in MRSA carriers in LTCFs [12].

Persistent MRSA carriers are more often colonized at multiple sites, are more likely to transmit to others, and become infected than transient carriers [24]. However, this aspect has not been studied in MRSA-colonized residents in LTCFs. A recent study performed in a LTCF showed that the degree of bacterial colonization in persistent MRSA carriers was significantly higher than in transient MRSA carriers [14]. We did not find a relationship between the incidence of MRSA infection and the duration of MRSA carriage, possibly because of the very few cases of MRSA infections. Moreover, the incidence of MRSA infection was similar in prevalent MRSA carriers and residents with newly acquired MRSA, i.e. MRSA colonization acquired while residing at the facility. A recent study, which included a small number of residents in LTCFs, found that the risk of MRSA infection in long-term carriers in the first year exceeded the risk of infection in subsequent years [23]. It appears that MRSA carriers remain at considerable risk for subsequent MRSA infection regardless of the time since the initial detection of MRSA carriage. Available data indicate that MRSA colonization in LTCFs may have different and less severe consequences than in acute-care hospitals. The risk of MRSA infection in the population of community LTCFs might not be related to the duration of colonization but might instead be attributable to known risks associated with MRSA infection such as hospitalization, bronchoaspiration, and the presence of decubitus ulcers or invasive medical devices. Except for ulcers and bronchoaspiration, these risks are not frequent in this population [24]. Reports of MRSA producing Panton–Valentine leukocidin (PVL) strains in LTCFs are increasing [25–27]. Since these strains might produce spontaneous infections, MRSA infection rates could potentially rise in residents in LTCFs

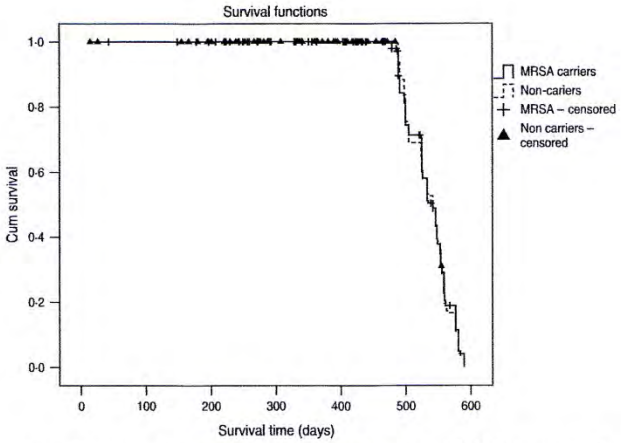

Fig. 1. Overall mortality during the study period (18 months) for cohorts of MRSA carriers and non-carriers.

without obvious clonal spread. None of the strains in this study was PVL positive [2] and previous molecular typing had shown the presence of only two distinct clones [16] one of which (CC5-MRSA-IV) has been reported as widely disseminated in Spanish hospitals [28, 29].

We found no differences in overall mortality in MRSA carriers and non-carriers. The mortality rate was around 15–20% in this elderly population and bronchoaspiration was the most frequent cause of death (data not shown). Previous studies have reported an associated mortality of MRSA infections in LTCFs of 1% [13], and a relative risk overall mortality rate of 2·0 in MRSA carriers [12]. Significantly higher mortality was associated with MRSA carriers in LTCFs only in patients with severe cognitive impairment [30].

This study has some limitations, as we only performed cultures of nasal swabs and decubitus ulcers to detect MRSA colonization. A recent study demonstrated that more than half of community LTCF residents present multiple-site MRSA colonization and one-third of MRSA carriers would have been missed if only nasal swabbing had been performed [31]. Another limitation is the large number of residents lost to follow-up, principally because of death in an elderly population. Patients lost to follow-up had significantly more deterioration in functional status; this is expected since poor functional status is associated with death in this population [32]. In addition this study was originally designed to describe the natural history of MRSA colonization in residents in LTCFs and to identify risk factors for being colonized with MRSA [16]. The MRSA infection rate and mortality in both cohorts were considered as secondary outcomes, and thus, no specific calculations were initially performed to determine if the study had sufficient power to detect significant differences. Nevertheless, major strengths of this study are the prospective design and the fact that it includes multiple facilities with a similar profile. Moreover, MRSA infections and mortality were evaluated in a population with a high prevalence of MRSA carriage.

Community LTCFs are institutions intended for the promotion of a healthy lifestyle for elderly people, a segment of population that is growing steadily; promoting comfort, optimal social environment and preserving functional status of residents are major objectives. The profile of community LTCFs and the endemicity of MRSA in these centres with a low clinical impact for colonized residents while in the facility, make the implementation of control measures to limit MRSA spread controversial. Standard precautions for all residents should be applied routinely; barrier precautions, cohorting, decolonization and other measures should be undertaken only for controlling MRSA infection outbreaks [33–36]. Our results together with the clinical experience and available literature suggest that MRSA infections are neither frequent nor severe while MRSA-colonized

residents remain in a LTCF. However, when admitted to an acute-care centre, they may spread MRSA to other patients who may develop severe infections. Therefore the epidemiological impact of the reservoir of MRSA in LTCFs is more relevant than the clinical impact of this colonization for an individual resident. The present results support current recommendations to control MRSA spread in community-LTCFs [33–36].

APPENDIX

Members of the Spanish Network for Research in Infectious Diseases who participated in the study: Raul Fernández, David Herrero, Rosario Casas, Eulalia Fontseca, Mónica Bota, Ricard Iniesta, Jesús Alburquerque, Catalina Andreu, Enrique Campos, Montserrat Vaqueiro, Esperança Antón, Jordi Trelis, Anna Esteve, Maria Canals, Ana Diaz, Eva Penelo, Antonio Oliver, Javier Ariza, Francesc Gudiol.

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DECLARATION OF INTEREST

None.

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